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COMBINED EFFECTS OF GIBBERELLIN AND 2,4-D ON DORMANT SEEDS OF STINKWEED (THLASPI ARVENSE L.)[1]

WILLIAM G. CORNS

Abstract

Concentrations of 50 and 500 p.p.m. gibberellin were used alone and in combination with 2,4-D sodium salt solutions ranging from 0.00001 to 8000 p.p.m. acid equivalent, in Petri dishes, with dormant seeds of stinkweed (*Thlaspi arvense* L.), to determine the effects of these treatments or of subtoxic amounts of 2,4-D alone, on germination and growth. About 90% germination within 2 weeks was induced by the 50 p.p.m. gibberellin and was not augmented by the very dilute concentrations of 2,4-D. Complete germination occurred within only 8 days with 500 p.p.m. gibberellin alone. Strengths greater than approximately 0.02 p.p.m. of 2,4-D retarded shoot elongation and concentrations greater than about 0.5 p.p.m. of 2,4-D progressively reduced germination associated with either 50 or 500 p.p.m. gibberellin. Concentrations of 2,4-D higher than approximately 200 p.p.m. prevented both concentrations of gibberellin from causing any true germination. Beyond 200 p.p.m. of 2,4-D there was still, however, a broad range within which gibberellin was able to cause a definite initial phase of the germination process characterized simply by rupture of the seed coat without protrusion of root and stem. This response could not be prevented by 2,4-D at concentrations lower than 4000 p.p.m. in counteraction with 50 p.p.m. gibberellin, or at dosage lower than about 7000 p.p.m. in association with 500 p.p.m. gibberellin. Potential practical significance of the work is considered.

Introduction

Effects of gibberellin upon the germination of a number of species of weed seeds at different times after their collection from plants growing under natural conditions at Edmonton have been reported by us (1). One of the most notable results was the consistent, virtually 100%, germination of completely dormant seeds of stinkweed shortly after the chemical treatment. The present work deals with the response of similar dormant stinkweed seeds to 2,4-dichlorophenoxyacetic acid (2,4-D) used in conjunction with gibberellin treatments. The subject appears to have both basic and practical implications. Gibberellin may be present as a normal constituent of higher plants (2), hence its interaction with other growth regulators is of considerable interest. Moreover, the selective herbicide 2,4-D is commonly sprayed for control of stinkweed in cereal crops. Occasionally seeds may be present on the weeds at spraying time or sometimes develop and mature subsequently. Additional

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Contribution from Division of Crop Ecology, Department of Plant Science, University of Alberta, Edmonton, Alberta.

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knowledge about removal of irregularities of seed behavior may help to lessen the problem caused by the carryover of dormant weed seeds in the soil. An economical chemical for use on plant or soil to overcome seed dormancy would be a boon. A formulation that could overcome dormancy and also kill the seedling would be an even more valuable weapon against weeds.

Materials and Methods

The supply of stinkweed seeds was collected from mature plants in a field at Edmonton on August 31, 1959, and stored at 5° C. Samples of this seed were taken periodically for the experiments between September 1959 and February 1960. In each experiment 50 seeds were placed on a filter paper disk in each Petri dish. Three replicates were used for each treatment. Aliquots of distilled water (5 cc), 2,4-D (sodium salt, Baker Chem. Co.), gibberellin solution (from 10% potassium salt, Nutritional Biochem. Corp.), or a mixture of gibberellin and 2,4-D, were pipetted into the respective dishes before they were placed, with lids on, within a humid, dark germinating cabinet held at 21° C. Except where stated otherwise, the duration of each test was 8 days for the material involving 500 p.p.m. gibberellin and 2 weeks with the seeds in 2,4-D alone, or 50 p.p.m. gibberellin.

The 50 p.p.m. concentration of gibberellin was employed to induce substantial, though incomplete, germination, and the 500 p.p.m. to cause complete germination of the seeds within the given period of time. With each solution of gibberellin there was employed a wide range of concentrations of 2,4-D as noted in Table I. Three or four concentrations were accommodated at a time in consecutive experiments and some of the tests were repeated at different times.

This series was designed to seek (*a*) a non-toxic concentration of 2,4-D that might induce germination of the seeds by itself or that might augment the stimulatory effect of the gibberellin; (*b*) the minimum concentration of 2,4-D that would have an inhibitory effect on responses to each of the concentrations of gibberellin respectively; (*c*) the minimum concentration of 2,4-D that would prevent or nullify responses from each of the concentrations of gibberellin respectively.

Results and Conclusions

During the course of the experiments it was possible to distinguish clearly three classes of seeds: (*a*) dormant—those that underwent no visible change apart from swelling due to absorption of water, (*b*) germinated—those with varying amount of seedling elongation beyond the testa, (*c*) ruptured, but not germinated—those whose coats broke open as a result of incipient expansion of cotyledons without further protrusion. Plate I illustrates representative material.

Where 2,4-D was used by itself at concentrations of 0.00001, 0.0001, 0.001, 0.01, and 10 p.p.m., there was no germination response during the observation period for any of these tests. The quantitive data for all of the other treatments are summarized in Table I.

During the experiments it was noted that germination in 50 p.p.m. gibberellin solution was slower than in the 500 p.p.m. concentration. This was noticeable within 2 days after the beginning of a test. The data in Table I show that by the end of 2 weeks the germination of seeds in the lower concentration approached that achieved in 8 days by seeds in the higher concentration of gibberellin.

TABLE I

Effects of 2,4-D with gibberellin on dormant seeds of stinkweed

2,4-D with 50 p.p.m. gibberellin (2 weeks)				2,4-D with 500 p.p.m. gibberellin (8 days)			
p.p.m. 2,4-D	% germ.	% testa rupture*	Seedling length, % of check†	p.p.m. 2,4-D	% germ.	% testa rupture*	Seedling length, % of check†
0	90‡	94	100	0	100‡	100	100
0.00001	85	94	105§	0.00001	99	100	90
0.0001	78	90	94	0.0001	100	100	101
0.001	91	96	105§	.001	100	100	93
0.005	97	100	103	.005	100	100	95
0.01	87	99	101	.01	100	100	98
0.02	78	88	90	.02	100	100	92
0.03	80	88	67¶	.03	99	100	72¶
0.04	74	84	60	.04	100	100	72
0.05	60	78	67	—	—	—	—
0.10	69	81	41	.10	91‖	100	31
0.20	68	88	27	—	—	—	—
0.30	66	87	22	—	—	—	—
0.40	71	86	—	—	—	—	—
0.50	73‖	89	—	.50	84¶	100	7
1	56¶	81	—	1	68	100	5
5	43	84	—	5	38	100	—
10	26	90	—	10	54	100	7
20	51	93	4	20	54	100	6
30	24	91	—	30	63	100	5
40	8	85	—	40	55	99	5
50	39	95	5	50	7	100	3
60	17	95	—	60	33	99	2
70	21	91	2—	70	15	100	2—
80	10	93		80	13	100	
90	9	91		90	5	99	
100	5	94		100	2	99	
150	2	98		150	1	100	
200	0	100		200	0	99	
250	3	95		250	0	100	
300	0	100		300	0	100	
400	0	99		400	0	100	
500	0	98		500	0	100	
1000	0	55		1000	0	96	
2000	0	31		2000	0	88	
3000	0	9		—	—	—	
4000	0	3		4000	0	25	
5000	0	0 (20 days)		5000	0	9	
				6000	0	3	
				7000	0	1 (2 weeks)	
				8000	0	0 (2 weeks)	

*Including germination %.
†10 longest seedlings per dish.
‡Representative figure from means of four experiments.
§Not statistically significantly greater than the control.
‖Significantly less than the control at 5% level.
¶Significantly less than the control at 1% level.

It has already been noted that there was no stimulation of germination and growth by 2,4-D alone in concentrations as low as .00001 p.p.m. or at higher concentrations. It can be seen that this was also true for 2,4-D in combination with either 50 or 500 p.p.m. gibberellin. Moreover, no greater concentration of 2,4-D was required for incipient reduction of germination and growth of seedlings in 500 p.p.m. gibberellin than for those in 50 p.p.m. gibberellin. Shoot elongation was retarded by concentrations in excess of about 0.02 p.p.m. of 2,4-D and germination percentage was progressively reduced by concentrations greater than approximately 0.5 p.p.m. of 2,4-D.

Concentrations of 2,4-D greater than about 250 p.p.m. prevented both concentrations of gibberellin from causing any "true" germination, i.e. visible elongation beyond the seed coat.

There was a striking difference between the amount of 2,4-D required to prevent germination in gibberellin solution and the much greater quantities (approximately 4000 p.p.m. of 2,4-D with 50 p.p.m. gibberellin and 7000 p.p.m. with 500 p.p.m. gibberellin) required to prevent the initial expansion processes from causing rupture of the seed coat (Plate I). Dormant seeds whose testas did not break did, nevertheless, absorb water from the medium to become moist, soft, and swollen as compared to the dry stock seeds. The rupturing of the seed coats of gibberellin-treated material was therefore not simply and solely dependent upon the ability of the seeds to take up water. It was associated with activity of gibberellin and could be counteracted by very high concentrations of 2,4-D.

The possible relationship between this response and other reported seed responses involving interaction between gibberellin, natural growth substances, and environmental factors (3) poses additional interesting problems. The significance of the results with respect to practical treatments of maturing plants is receiving attention and will be reported later.

Acknowledgments

It is a pleasure to acknowledge the keen interest and careful work of Research Technician Raymond Schraa and the financial assistance of the University of Alberta and of the National Research Council of Canada.

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PLATE I

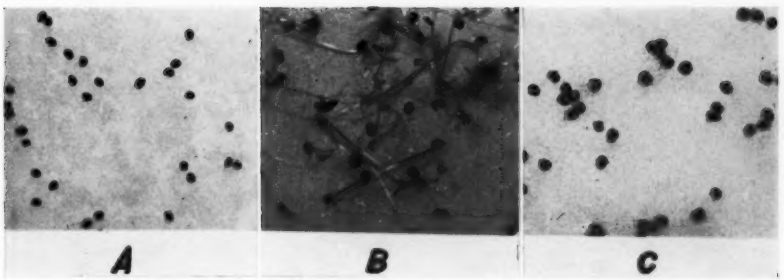

FIG. 1. (*a*) Control: dormant stinkweed seeds after 8 days incubation on filter paper moistened with water. (*b*) Gibberellin-treated seeds. (*c*) Gibberellin plus 2,4-D-treated seeds (see text for details).

EFFECTS OF KINETIN AND BENZIMIDAZOLE ON THE GROWTH OF ETIOLATED PEA STEMS AND BARLEY COLEOPTILES[1]

F. R. FORSYTH[2] AND D. J. SAMBORSKI[3]

Abstract

Optimum growth of etiolated pea stem sections was obtained in a basal solution of 3-indoleacetic acid, cobalt chloride, and sucrose. The final length of pea stem sections was increased when sucrose was added 2, 4, or 6 hours after 3-indoleacetic acid and cobalt were added to the test solution. Benzimidazole at 100 to 200 p.p.m. and kinetin at 1 p.p.m. added to the basal solution inhibited growth of etiolated pea stem sections. Growth of the pea stem sections was inhibited when they were treated for 4 hours with kinetin plus 3-indoleacetic acid, then transferred to the basal solution for 14 hours. There was no growth inhibition when pea stem sections were treated for 4 hours with kinetin alone, followed by 14 hours on the basal solution. Similar results with kinetin were obtained when barley coleoptiles were used and 2,4-dichlorophenoxyacetic acid was the growth-promoting substance. Ether and hot water extracts of pea stem tissue pretreated with kinetin and 3-indoleacetic acid did not appear to contain a growth inhibitor.

Introduction

Detached wheat leaves, when floated for relatively long periods on appropriate concentrations of BENZ[4] or KIN, show no appreciable changes in levels of protein, amino acid, or soluble carbohydrate, and their reactions to leaf and stem rusts are the same as those of attached leaves (9, 11). The mechanisms by which BENZ and KIN act in maintaining protein integrity and normal rust reactions are of considerable interest to research workers in protein metabolism and in host–parasite relations. In this work the activities of the two compounds were studied using sections of pea stems and barley coleoptiles.

The etiolated pea stem section test has been used widely (1, 4, 5, 6, 8, and 10) in studying the responses of plant tissue to IAA. This test (12), and a modified test using light-grown, dwarf-pea stem sections (2, 3), have also been used to show interactions between GA, KIN, and other growth substances. The barley coleoptile test has been used to measure the phytotoxicity of various chemicals (7). These tests were used in this study to investigate the interactions between KIN, BENZ, CO, IAA, GA, and S.

Methods

Pea seeds (*Pisum sativum* L. var. Alaska) were soaked for 3 hours in distilled water, sown in quartz sand in 250-mm crystallizing dishes, and kept

[1]Manuscript received May 30, 1960.
Contribution No. 55 from the Canada Department of Agriculture Research Station, Winnipeg, Manitoba.
[2]Formerly at Plant Pathology Laboratory, Winnipeg; now at Pesticide Research Institute, London, Ontario.
[3]Plant Pathology Laboratory, Winnipeg.
[4]Abbreviations used in this paper: BENZ for benzimidazole; KIN for kinetin; CO for cobalt chloride hexahydrate; IAA for 3-indoleacetic acid; GA for gibberellic acid; S for sucrose; 2,4-D for 2,4-dichlorophenoxyacetic acid.

in a dark room. The seedlings were watered every second day; they were ready for use after 7 to 8 days' darkness at 23–25° C. Preliminary investigations showed that a 5-mm section of the third internode, between 1 and 6 mm from the apical crook (see Fig. 1), gave uniform results. The apical crook was snapped off with forceps and the 5-mm section was removed, using two razor blades mounted 5 mm apart on a metal bar. This operation was carried out under a phototropically inactive red light. Ten such sections were placed on Whatman No. 1 filter paper in each Petri dish (60 mm diameter), together with 5 ml of the test solution. After 18 hours in darkness on the test solutions the sections were measured to the nearest 1/10th of a millimeter. For studies with coleoptile sections of barley (*Hordeum vulgare* L. var. Montcalm), the methods developed by Ludwig *et al.* (7) were followed.

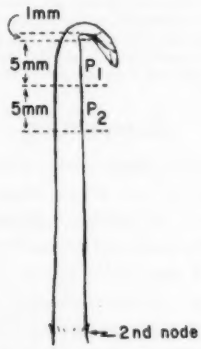

FIG. 1. Apex of dark-grown 7- to 8-day-old pea plant showing location of P_1 and P_2 sections.

For extraction of a growth inhibitor the pea seedlings were grown in the dark, as described above, and when the third internodes were 1–3.5 cm in length they were excised and the apical crooks were removed. The internodes were then divided into two lots, one of which (13.4 g fresh weight) was placed on filter paper moistened with a phosphate buffer solution (0.01 *M*; pH 5.5) containing IAA (5 p.p.m.) and KIN (2 p.p.m.), and the other (11.0 g fresh weight) was placed on filter paper moistened with buffer only. After 5½ hours the internodes were washed with distilled water, blotted dry, and weighed. Each lot was then extracted three times with cold ether for half an hour each time. The extracts were combined and evaporated to dryness. The residue was then taken up in 1 ml of ether (peroxide free) and used for chromatography. Following the ether extractions, the pea stem sections were also extracted with hot distilled water. The water extracts were evaporated under reduced pressure at 35° C. The residue was taken up in 1 ml of water and also used for chromatography. For the chromatograms a 50 μl aliquot of each extract was spotted on Whatman No. 3 paper, along with marker spots of IAA, KIN, and IAA plus KIN; a 10:1:1 mixture of isopropyl alcohol, con-

centrated NH_4OH, and water was used as the solvent. The papers were cut longitudinally into 1-in. strips, from which 2-in. sections (including the 1 inch preceding the origin) were cut for bio-assay.

Results

A preliminary experiment showed that the P1 section (cf. Fig. 1) was more responsive than the P2 section to IAA, CO, and S, a result which agrees with that of Purvis and Hillman (10). Accordingly, P1 sections were used in all subsequent work.

Brian and Hemming have reported that KIN inhibits IAA induced elongation of light-grown, dwarf-pea stem sections (2). The data of Table I show the effects of BENZ and KIN on the elongation of etiolated pea stem sections. KIN and BENZ did not completely inhibit the stimulatory effects of IAA, CO, and S, since some elongation occurred in their presence; between 100 and 200 p.p.m. BENZ was required to cause as much inhibition as 1 p.p.m. KIN.

TABLE I

BENZ and KIN as inhibitors of growth in etiolated pea stem sections

Treatment (18 hours)	p.p.m.	Av. length of sections (mm)
IAA + S + CO + BENZ	200	7.08
+	100	8.84
+	50	9.28
+	20	9.19
+	10	9.47
+ KIN	1	7.70
IAA + S + CO		9.85
Distilled water		6.21
	LSD 5%	0.53
	LSD 1%	0.74
	LSD 0.1%	1.01

NOTE: IAA was used at 1 p.p.m., S at 2%, $COCl_2.6H_2O$ at 8×10^{-6} *M*, all in 0.01 *M* phosphate buffer at pH 5.5.

Brian and Hemming concluded that GA and CO increase the extension growth of dwarf-pea stem sections in the presence of IAA, and that the increases are independent and additive (3). The data of Table II show that, in the presence of IAA and S, GA gives about the same stimulation of the sections as does CO, and that when both are present they give greater stimulation than does either singly. The data also show that, with IAA present, KIN inhibited the stimulation induced by GA to the same extent as that induced by CO.

During these experiments it was also found that elongation of the pea stem sections in the presence of IAA, CO, S, and KIN or BENZ was dependent on when the S was added. The data of Table III show that there was an increase in the final length of the sections when the addition of S was delayed up to 6 hours. In certain experiments, not shown in Table III, a stimulatory effect was given by very low (0.001 p.p.m.) concentrations of KIN, but this result was not readily reproducible. Low concentrations of BENZ gave no stimulation.

TABLE II

KIN as an inhibitor of growth in etiolated pea stem sections in the presence of CO or GA

Treatment (18 hours)	Av. length of sections (mm)
IAA + S + GA	9.8
S + GA	8.5
GA	7.7
S	6.9
IAA	8.7
IAA + S	9.0
IAA + GA	9.1
IAA + S + CO	10.2
IAA + S + CO + KIN	7.7
IAA + S + CO + GA	11.5
IAA + S + GA + KIN	7.8
H_2O	6.6
LSD 5%	0.35
LSD 1%	0.48
LSD 0.1%	0.64

NOTE: IAA was used at 1 p.p.m., S at 2%, GA at 10 p.p.m., CO at 8×10^{-5} *M* $COCl_2.6H_2O$, KIN at 1 p.p.m., all in 0.01 *M* phosphate buffer at pH 5.5.

TABLE III

The effect of time of addition of S on the growth of etiolated pea stem sections

Treatment	No sucrose added	Sucrose added 0 time	Sucrose added 0 + 2 hr	Sucrose added 0 + 4 hr	Sucrose added 0 + 6 hr
	Average length of sections (mm)				
IAA + CO	8.4	7.9	8.9	9.5	10.0
IAA + CO + .001 p.p.m. KIN	8.4	7.8	8.6	9.3	9.4
IAA + CO + 2.0 p.p.m. KIN	7.1	6.6	7.3	7.5	7.4
IAA + CO + 0.1 p.p.m. BENZ	8.2	7.6	8.9	9.5	10.0
IAA + CO + 10.0 p.p.m. BENZ	8.2	7.6	8.6	9.4	9.3
IAA + CO + 100.0 p.p.m. BENZ	8.2	7.4	8.5	8.9	9.5

NOTE: IAA was used at 1 p.p.m., S at 2%, and CO at 8×10^{-5} *M* $COCl_2.6H_2O$, all in 0.01 *M* phosphate buffer at pH 5.5.

Brian and Hemming state that KIN alone does not inhibit extension growth unless relatively high concentrations (10 μg per ml) are used, but that if IAA is present, KIN gives strong inhibition of extension growth, even at low concentrations (2). They consider that in the presence of both KIN and IAA a growth inhibitor may be produced. From the data of Table IV it is clear that both KIN and IAA must be present to inhibit the elongation of etiolated pea stem sections. A 4-hour pretreatment with KIN and IAA before transfer of the sections to IAA, CO, and S resulted in an inhibition of stem elongation, whereas a 4-hour pretreatment with KIN alone did not. In these experiments the pea stem sections were washed thoroughly in distilled water and blotted dry between treatments, so that there was little or no carry-over of chemicals from one treatment to the next.

TABLE IV

The effect of time of addition of KIN and BENZ in relation to IAA on the inhibition caused by KIN and BENZ to etiolated pea stem sections

Treatment		Av. length (mm)	Fresh weight /10 sections (mg)
0–4 hours*	5–18 hours*		
IAA + BENZ 100 p.p.m.	IAA + CO + S	8.8	24.2
IAA + BENZ 200 p.p.m.	"	8.5	24.6
IAA + CO + S	"	8.7	25.0
IAA + CO	"	8.6	24.0
IAA	"	8.8	23.5
CO	"	9.4	26.2
IAA + CO + S + KIN 1 p.p.m.	"	7.5	25.5
IAA + KIN 1 p.p.m.	"	6.9	23.8
KIN 1 p.p.m.	"	9.1	25.1
Buffer	"	9.3	24.5
IAA	IAA	7.8	18.2
KIN	KIN	6.2	15.1
Buffer	Buffer	6.6	15.3

NOTE: IAA was used at 1 p.p.m., CO as cobalt chloride at 8×10^{-5} *M*, all in 0.01 *M* phosphate buffer at pH 5.5.
*The sections were placed for 4 hours on the solutions indicated in the 0–4 hour column, then transferred to the solutions indicated in the 5–18 hour column for the duration of the experiment.

TABLE V

The effect of time of addition of KIN in relation to 2,4-D on the inhibition caused by KIN to etiolated barley coleoptile sections

Treatment		Increase in length of sections (mm)	% inhibition
0–4 hours	5–18 hours		
2,4-D + S	2,4-D + S	15.4	—
KIN + S	2,4-D + S	15.6	0
KIN + 2,4-D + S	2,4-D + S	13.5	12.6
KIN + S	KIN + S	11.8	23.2
KIN + 2,4-D + S	KIN + 2,4-D + S	9.7	37.2

NOTE: KIN was used at 2 p.p.m., 2,4-D at 1 p.p.m., S at 1%, and *M*/12 phosphate buffer at pH 6.2.

In the presence of IAA and S there was an increase in the diameter of KIN-inhibited sections over that of the non-inhibited sections; the fresh weight of the short inhibited sections was little different from that of the elongated sections (Table IV). Although KIN inhibits elongation, it does not appear to hinder the uptake of water. In the presence of IAA and S, the 4-hour BENZ pretreatment inhibited neither elongation nor water uptake.

The data of Table V show that 2,4-D stimulated elongation of barley coleoptile sections is inhibited by a 4-hour pretreatment with KIN, and that KIN is not inhibitory when 2,4-D is absent. The effect of KIN in inhibiting elongation of both pea and barley sections is thus expressed only when a growth-promoting substance is present.

If KIN inhibits elongation by depressing either the uptake or the activity of exogenously supplied IAA or 2,4-D, it may be expected that addition of IAA or 2,4-D in excessive amounts will overcome the KIN-induced inhibition.

TABLE VI

The effect of KIN on elongation of barley coleoptile sections in a medium of buffer + S + 2,4-D or IAA

KIN (p.p.m.)	2,4-D (p.p.m.)			IAA (p.p.m.)			
	8	4	2	8	4	2	0
	Increase in length of sections in mm						
10	17.7	18.1	17.9	16.5	16.5	16.0	16.1
5	18.3	17.7	17.8	16.4	16.8	17.3	16.1
2.5	17.3	17.5	17.1	17.6	16.1	17.6	15.9
1.25	18.9	19.0	17.6	17.4	17.5	16.0	15.7
0.63	18.5	18.2	17.8	17.2	17.1	16.9	14.5
0	—	20.3	—	—	—	—	—

NOTE: Each test solution contained, in addition to the above chemicals, *M*/12 phosphate buffer at pH 6.2 and S at 1%.

The data of Table VI show that concentrations of KIN from 0.63 to 10 p.p.m., when added to the test medium, inhibited the elongation of barley coleoptile sections in the presence of either IAA or 2,4-D at concentrations of 2 to 8 p.p.m. The inhibitory effect of even the lowest concentration of KIN was not decreased by the addition of IAA or 2,4-D.

It is possible that the inhibition which occurs when both KIN and IAA are present is due to the formation of a new growth-inhibitory compound. In an attempt to isolate such a compound, water and ether extracts were taken from pea stem sections that had been incubated on a solution containing IAA (5 p.p.m.) and KIN (2 p.p.m.). The extracts were chromatographed to separate a possible inhibitor from free IAA and KIN, and 1-in. wide paper strips were cut into 8 sections. Pea stem sections were incubated for 4 hours on each section of filter paper (eluted with 5 ml of phosphate buffer), then transferred to IAA, CO, and S, for 14 hours. This experimental procedure results in inhibited growth of pea stem sections when the 4-hour incubation medium contains IAA and KIN. Similar growth inhibition should be obtained after incubation with an inhibitor, if one is produced. The results gave no evidence of such a compound in either the ether or the water extracts. It may be that the hypothetical compound is highly unstable; in any case the amount of plant material used was insufficient to warrant any definite decision on presence or absence of an inhibitor.

Discussion

KIN has the effect of preventing the normal response of plant tissues to IAA in several biological systems. Examples of this include the removal of auxin-induced inhibition of lateral bud development (12), the inhibition of extension growth of light-grown, dwarf-pea stem sections in the presence of IAA (2), and the prevention of the seedling response of light-grown dwarf peas to GA (2). It is clear that growth inhibition results from an interaction of KIN and a growth-promoting substance. However, the mechanism of

this inhibitory interaction is not known. It is possible that the transport of KIN to its site of inhibitory action is facilitated by the growth-regulating compound, or that in the presence of KIN a new compound is formed. Our results do not rule out the possible formation of such a new compound.

KIN and BENZ exert a profound influence on the maintenance of protein in detached leaves. In view of the observations with pea stem sections, it seems possible that an IAA–KIN or an IAA–BENZ complex takes part in the process, with a definite ratio of IAA to KIN or BENZ (or other compounds with similar activity) as a requirement. However, this concept must be treated with reservation. While there is strong evidence for an IAA–KIN interaction, our results do not show any interaction between IAA and BENZ. It is therefore possible that KIN and BENZ act quite differently in maintaining protein levels of detached leaves.

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STUDIES ON SUNFLOWER RUST

II. LONGEVITY OF UREDIOSPORES OF PUCCINIA HELIANTHI[1]

W. E. SACKSTON[2]

Abstract

Urediospores of *Puccinia helianthi* Schw. collected from sunflower plants in the field and stored in glass vials at 5° C in a refrigerator survived for 846 days. In a freezer at −10° to −22° C their ability to infect plants and to germinate on agar appeared unimpaired after 1652 days' storage. Storage under mineral oil decreased spore longevity at both refrigerator and freezer temperatures. Viable spores dispersed in oil germinated well on agar and induced heavy infections on sunflowers.

The longevity of dry spores of *P. helianthi* stored at −10° C to −22° C and the high level of viability retained greatly exceed those reported for other species of rusts.

Research on sunflower rust (*Puccinia helianthi* Schw.) at Winnipeg (6, 9) necessitates the maintenance of many cultures of rust for long periods of time. As high summer temperatures make it difficult to keep rust cultures alive on sunflower plants in the greenhouse during July and August, urediospores have been stored during the summer in vials in a refrigerator at about 5° C with excellent results. Storage for even a few months reduces the labor and time required to keep rust collections viable. By reducing the number of spore generations each year on the host, and also the number of collections which must be grown in the greenhouse at any one time, storage also reduces the danger of contamination of one race by another. Storage for longer periods of time would multiply the benefits.

A great deal of work has been done on the conservation of fungous cultures. In a recent review, Fennel (2) listed over 300 papers on the subject, but very few of them dealt with rusts. Maneval (5) investigated the longevity of urediospores of several species of rusts, including *P. helianthi*, at room temperature and in an ice chest. Sharp and Smith (11, 12) lyophilized urediospores of cereal rusts, and found that they survived four times as long as spores in cold storage. Haskins (4) lyophilized urediospores of several species; *P. helianthi* lyophilized before storage germinated about 40%, whereas unlyophilized control samples germinated only about 15%.

A method of storing urediospores for long periods without lyophilization or other preparation would be very useful for any rust species which could survive such treatment. Akai (1) stored urediospores of wheat leaf rust (*P. triticina* Eriks.) at −10° C; germination declined rapidly after 1 day of storage, and was nil after 60 days. Flor (3) found that the germination of urediospores of flax rust (*Melampsora lini* (Pers.) Lév.) stored in small vials in a food freezer at −10° C persisted as a trace for more than 3½ years. His success prompted the experiments with urediospores of *P. helianthi* reported in this paper.

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Materials and Methods

Urediospores collected from sunflower plants in the field in 1955 were placed in small vials, about 3 to 4 cc per 7-cc vial, stoppered tightly with corks, and were stored in a food freezer kept at about −10° C to −15° C in a private home.

Urediospores collected from sunflowers in the field in July and August, 1956, were stored in vials under various conditions. Some of the vials were stored at laboratory temperature, between 20° and 30° C; some were stored in the refrigerator compartment of a combination refrigerator–freezer, at about 5° C; and others were kept in the freezer compartment, at about −10° to −22° C. Four vials of each of two 1956 field collections of spores were placed in each location without any treatment ("dry"). Other vials of each collection had sterile mineral oil, medicinal grade, added to cover the spores to a depth of 1 cm ("oil") and were then placed at the respective temperatures. Spores stored dry and under oil at the various temperatures were removed at intervals and used to inoculate sunflower plants in a greenhouse. The germination on water or on agar of spores from the respective vials was also determined at intervals.

Results and Discussion

The results of inoculations with urediospores stored under various conditions for intervals up to 1273 days are given in Table I. The results of inoculations with two other lots of spores from storage are also given in the table. It had been planned to make inoculations at monthly intervals for the first 6 months. As the first inoculations were not made until after 110 days of storage, however, it was not possible to determine the rate of loss of viability of the spores.

Infection of sunflower seedlings by urediospores stored dry more than 2 years at 5° C in a refrigerator indicates that the longevity of *P. helianthi* under these conditions greatly exceeds that of other species of rust (12), as well as the value of 187 days for *P. helianthi* reported by Maneval (5). Survival does not depend on the storage of small quantities of spores in vials. Since these experiments were started, quantities up to 300 cc of urediospores stored in jars in a refrigerator for a year have been used successfully in inoculating sunflower plants in field plots.

Storage of urediospores of *P. helianthi* dry in a freezer at −10° to −22° C for almost 5 years apparently has had no adverse effect on the ability of the spores to infect sunflowers. As indicated in Table II this long storage has also had no apparent effect on the germination of the spores on agar. The volume of spores stored does not seem to affect their longevity. Quantities up to 200 and 300 cc of spores in jars have been stored in the freezer for more than 2 years with no observed loss in viability.

The remarkable longevity of sunflower rust spores in the freezer was quite unexpected. Flor (3) found that the germination of flax rust spores fell off sharply after 1 day in the freezer, and remained relatively stable at about

5% for 3 months. It then declined gradually to 1% after 1 year, and persisted as a trace for more than 3½ years. As there has been no apparent decline in the infectivity or germination of sunflower rust spores after 4½ years of storage, it may be necessary to continue the experiment for many years to determine their maximum survival time.

TABLE I

Infection of sunflower seedlings by urediospores of *Puccinia helianthi* stored in various ways since 1956

Storage, days	Room temperature		Refrigerator		Freezer	
	Dry	Oil	Dry	Oil	Dry	Oil
0	++++*	++++	++++	++++	++++	++++
110	+	+	++++	+++	++++	++++
125	0	0	—	—	—	—
180	±	0	++++	++++	++++	+
334	—	—	+++	++	+++	+++
491	—	—	++++	±	+++	++
540	—	—	++++	0	++++	+++
581	—	—	+++	±	++++	+++
754	—	—	++	0	++++	++
846	—	—	±	0	++++	+
897	—	—	0	0	+++	+
1038	—	—			++++	±
1273	—	—			++++	+
Field collection stored since 1955						
1652	—	—	—	—	++++	—
Greenhouse collection stored since 1955†						
1543	—	—	—	—	±	—

*Infection ratings: ++++ very heavy, +++ heavy, ++moderate, + light, ± one or two scattered pustules, 0 no infection.

†Rust collected in greenhouse December 1955 was stored in refrigerator until May 1956, then transferred to freezer.

TABLE II

Germination (%) of urediospores of *Puccinia helianthi* stored in various ways since 1956

Storage, days	Room temperature		Refrigerator		Freezer	
	Dry	Oil	Dry	Oil	Dry	Oil
2	75–80	—	—	—	—	—
65	10–15	65	10–15	80–85	82	10–15
110	0	0	19	95	55	35
131	0	0	—	—	—	—
897	—	—	0	0	60	25–50
1321	—	—	—	—	60–69	—
Field collection stored since 1955						
1687	—	—	—	—	78	—
Greenhouse collection stored since 1955*						
1575	—	—	—	—	0	—

*Rust collected in greenhouse December 1955 was stored in refrigerator until May 1956, then transferred to freezer.

Field collections of sunflower rust spores did not survive long periods of storage at laboratory temperatures, 20° to 30° C. Covering the spores with oil increased their percentage germination after 65 and 110 days of storage, as shown in Table II, but it did not increase their survival time. The increase in germination may have been due to better dispersal of the spores from oil and reduced crowding in the water drops in which germination was determined. Spores stored under oil in the refrigerator and in the freezer remained infective for much shorter periods than those stored dry (Table I). The cause of this reduction in longevity was not determined.

Rowell (7) showed that germination of cereal rust spores desiccated by long storage could be improved by dispersing them in oil to slow their rehydration on the germination medium. This same effect was demonstrated with sunflower rust spores which had been stored dry in the freezer for 897 days. When spores were placed on agar directly upon removal from the freezer, their germination was 60%; when they were dispersed in oil before being placed on agar, their germination was 88%. When some of the same samples of spores were used to inoculate sunflower seedlings, excellent infections were obtained both with the spores applied dry and with those dispersed in oil; this agrees with the results obtained with cereal rust spores by Rowell and Hayden (8).

Oil has been used successfully to conserve cultures of many fungi on agar (2), but apparently it has not been tried previously for this purpose with rust urediospores. Lack of success with sunflower rust should not prevent experimentation with other species. The demonstrated usefulness of oil as a dispersing medium in germination tests and inoculations would enhance its value as a storage medium for any rust species for which it could be used, particularly where expensive facilities were not available.

Sharp and Smith stated that spores of oat rusts collected from plants in the field stored better than spores collected in the greenhouse (12). This may be true also for sunflower rust. Spores collected from sunflowers in the greenhouse and stored in the freezer for 1151 days germinated well and induced heavy infections. However, another collection of spores from greenhouse plants, stored for 1543 days, produced an average of only one pustule on each two plants inoculated, and apparently failed to germinate on agar. The longevity of this particular sample may have been affected by storage in a refrigerator for 6 months immediately after collection, until a freezer became available at the laboratory.

Spores stored at laboratory temperatures and in the refrigerator continued to induce infections when inoculated onto sunflowers, long after their germination percentage on agar dropped to zero. This discrepancy may be explained most simply on the basis of the numbers of spores involved. Germination percentage is usually determined by counting several hundred or sometimes up to two thousand spores. Many thousands of spores are customarily applied to each inoculated leaf. Therefore, there is a much greater probability that one viable spore in several thousand could induce an infection than that its germination would be observed under the microscope.

One of the advantages of storing spores directly in a freezer over lyophilization was indicated in Flor's work (3). Each time that he removed a vial of flax rust urediospores from the freezer to make inoculations and to test germination the spores were warmed to room temperature, then cooled again to −10° C. They survived this alternate thawing and freezing 36 times. The vials of urediospores of *P. helianthi* used in the tests reported here were removed from the freezer for 1 to 2 hours each time they were used to inoculate plants or in germination tests. They survived thawing and refreezing 14 times in 3½ years with no apparent loss of viability. In contrast, Sharp and Smith (12) found that lyophilized urediospores of oat rusts died if the lyophil tubes were cracked during storage. Although spores of oat rusts in opened lyophil tubes could not be stored for long periods, they remained alive for at least 2 weeks if the opened tubes were kept in desiccators (12), making it possible to use the same tube for a period of days or weeks.

An experiment was made to determine the longevity at room temperature of urediospores of sunflower rust which had been stored in the freezer. This information would be important in estimating the probable effect on the viability of frozen spores of prolonged exposure to room temperature which might result from a power failure, shipment for study at some distant location, or other cause. A vial of spores which had been in the freezer for over 3½ years (1339 days) was transferred to a laboratory bench. Spores were removed from the vial at weekly intervals to inoculate sunflower seedlings, and at less regular intervals to determine the percentage germination on agar. The results are given in Table III. The ability to induce infection persisted at a fairly high level longer than did ability to germinate on agar; this agrees with the results presented in Tables I and II.

TABLE III

Survival of urediospores of *Puccinia helianthi* at room temperature after long storage in a freezer

Days at room temperature	Germination on agar, %	Infection of sunflower seedlings in greenhouse
(After 1339 days in freezer)		
2	61	Very heavy
9	—	Heavy
18	8	Heavy
25	5	Heavy
32	3	Very heavy
38	2	Moderate
45	0.1	Moderate
52	0.2	Light
59	0	Trace
66	0	Scarce (only two pustules on seven plants)
(After 1371 days in freezer)		
7	50	
13	37	
20	53	
27	11	
34	4	
41	2	
48	0	

A second vial was transferred from the freezer to laboratory temperature 32 days after the first (after 1371 days of storage) and spores were taken regularly at weekly intervals to follow the rate of decline of germination. The results, given in Table III, are conspicuously different from those obtained with the earlier sample. The first test was started late in March, the second in April. It is probable that uncontrolled variations in temperature and humidity in the laboratory during the two periods were sufficient to account for differences in the survival of the spores.

Spores removed from the freezer and stored at room temperature longer than 3 weeks showed somewhat reduced vigor as well as lower viability. They germinated more slowly than spores taken directly from the freezer, and, by the time that germination had fallen below 1%, produced shorter, weaker germ tubes. They also required about 1 day longer than spores fresh from the freezer to induce flecking and to produce sporulating uredia on inoculated sunflower plants in the greenhouse. This decreased vigor was not transmitted to the next spore generation. Spores collected from the slowly developing uredia germinated and induced infection as vigorously and in the same time as other fresh spores.

Conclusions

The results presented in this paper demonstrate the usefulness of a food freezer for storing urediospores of sunflower rust for periods of several years. They may have additional significance. Urediospores of *P. helianthi* not only survived for much longer periods of time at 5° C and at −10° to −22° C than did other species of rusts (1, 3, 4, 12), but the level of viability retained was also very much higher. It may therefore be rewarding to make detailed comparative studies of the urediospores of *P. helianthi* and other species to determine if the differences in their ability to survive indicate differences in their physiology or biochemical constitution.

It may also be rewarding to speculate on the usefulness to sunflower rust of its remarkable tolerance to low temperatures. Sunflowers and sunflower rust are both thought to have originated in North America (10). It is not known how the rust spread from North America to attack sunflowers almost everywhere in the world (6). If that spread between continents or between countries involved long-distance travel in air masses at high altitudes and at low temperatures then the urediospores seem well adapted to withstand the rigors of such air travel. This may be particularly pertinent in considering the source of sunflower rust in Chile (10). To have come from Argentina, where it is often plentiful, the rust would have to be able to survive aerial transport over the Andes, which rise to 20,000 ft and higher between the two countries.

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THIAMINE REQUIREMENT OF PULLULARIA PULLULANS (DE BARY.) BERKHOUT[1]

E. W. B. WARD

Abstract

Growth of *Pullularia pullulans* in shake culture on a synthetic medium, composed of D-glucose, *l*-asparagine, and mineral salts, was shown to be dependent on a supply of thiamine. The thiamine could be replaced by a combination of equivalent amounts of thiazole and pyrimidine, but not by either alone.

Introduction

In spite of the repeated isolation of *Pullularia pullulans* from widely different locations, only a few studies have been made of its nutritional requirements. These have been concerned largely with the utilization of carbon and nitrogen compounds or with growth on complex organic substrates (1, 5, 6). Recently Bernier (2) and Clark and Wallace (3, 4) grew *P. pullulans* in laboratory culture in order to study various metabolic activities of the fungus. Yeast extract or similar complex organic nutrient was usually included in the culture medium in these investigations and Bauer (1) reported that growth was poor on media low in 'bios'. Schopfer and Blumer (8), however, reported that *Dematium pullulans* was not stimulated by thiamine in a medium composed of mineral salts, asparagine, and dextrose.

In this paper experiments are described which demonstrate the necessity of thiamine for growth of an isolate of *P. pullulans* in a synthetic medium.

Materials and Methods

The Isolate

An isolate of *P. pullulans* was obtained as a contaminant in vitamin-enriched media in shake culture flasks. It was found to grow particularly well in shake culture where it produced masses of budding conidia. For experimental purposes a stock culture was maintained in shaken 200-ml Erlenmeyer flasks containing 50 ml M.Y.G. medium (see below). Transfer of conidia to fresh media was made every 6 days.

Media

A rich organic medium (M.Y.G.) was routinely employed for the maintenance of stock cultures. It had the following composition: malt extract (Difco) 5.0 g, yeast extract (Difco) 5.0 g, D-glucose 15.0 g, and distilled water 1 liter.

A synthetic medium, which, unsupplemented with vitamins, did not support growth of the fungus, was used for experimental purposes. This was composed of: major nutrients—D-glucose 15.0 g, *l*-asparagine 2.36 g, KH_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.5 g; minor nutrients—$Fe_2(SO_4)_3$ 0.715 mg, $ZnSO_4.H_2O$ 0.540 mg, $Co(NO_3)_2.6H_2O$ 0.245 mg, $MnCl_2.4H_2O$ 0.135 mg, $H_2MoO_4.H_2O$

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0.085 mg, $CuSO_4.5H_2O$ 0.195 mg, $CaCl_2$ 4.0 mg. The pH was adjusted to 6.0 with *N* KOH and the total volume made up to 1 liter with distilled water. The minor nutrients were made up in a stock solution such that the inclusion of 1 ml in 1 liter of medium gave the required concentrations.

The following vitamins and components were used to supplement the above medium, thiamine-HCl 100 μg/l., pyridoxine-HCl 100 μg/l., Ca-pantothenate 100 μg/l., *d*-biotin 10 μg/l., and the two thiamine moieties thiazole (4-methyl-5-(2-hydroxylethyl) thiazole) 50 μg/l. and pyrimidine (4-amino-5-aminomethyl-2-methylpyrimidine.2HCl) 50 μg/l. These were added to the synthetic medium from stock solutions of 1000× the required concentrations.

Fifty-milliliter aliquots of the media were dispensed into 200-ml Erlenmeyer flasks fitted with loose aluminum foil covers. They were sterilized by autoclaving for 9 minutes at 121° C.

Inorganic chemicals were of reagent grade and biochemicals were of the best grade commercially available. All glassware was cleaned by washing in detergent and rinsing in tap water, rinsing in chromic acid solution, and rinsing in tap water followed by distilled water.

Inoculum

The inoculum was prepared from 6-day-old shake cultures grown on M.Y.G. at 15° C. The medium was removed by centrifuging at 3000 r.p.m. in a Servall Superspeed centrifuge for 10 minutes and discarding the supernatant. The conidia from one culture were then suspended in approximately 40 ml sterile distilled water, recentrifuged, the supernatant was discarded, and the conidia resuspended in 100 ml of the unsupplemented synthetic medium. Fifty-milliliter aliquots of this suspension were transferred to each of two 200-ml Erlenmeyer flasks and shaken for 24 hours at 15° C. The medium was then removed from these suspensions as before and the conidia were again suspended in 100 ml of the synthetic medium. The density of cells was determined with a haemacytometer and 0.5 ml of the suspension was used as inoculum for each culture flask.

Inoculation and Measurement of Growth

Experimental cultures were incubated in the dark at 15° C for 5 days on a Gyrotary shaker* with a radius of motion of 1 inch operating at approximately 275 r.p.m. Conidia were harvested in previously weighed Pyrex centrifuge tubes by centrifuging as described above. The supernatant was discarded and the conidia were washed by suspending in sterile distilled water, centrifuging, and discarding the supernatant a second time. The tubes and conidia were then dried for 24 hours at 90° C and the dry weights determined. The values reported below are the mean weights of three replicate cultures.

Results and Discussion

P. pullulans made comparable growth on the synthetic medium supplemented with a mixture of the four vitamins, thiamine-HCl, pyridoxine-HCl,

*New Brunswick Scientific Co., New Brunswick. N.J.

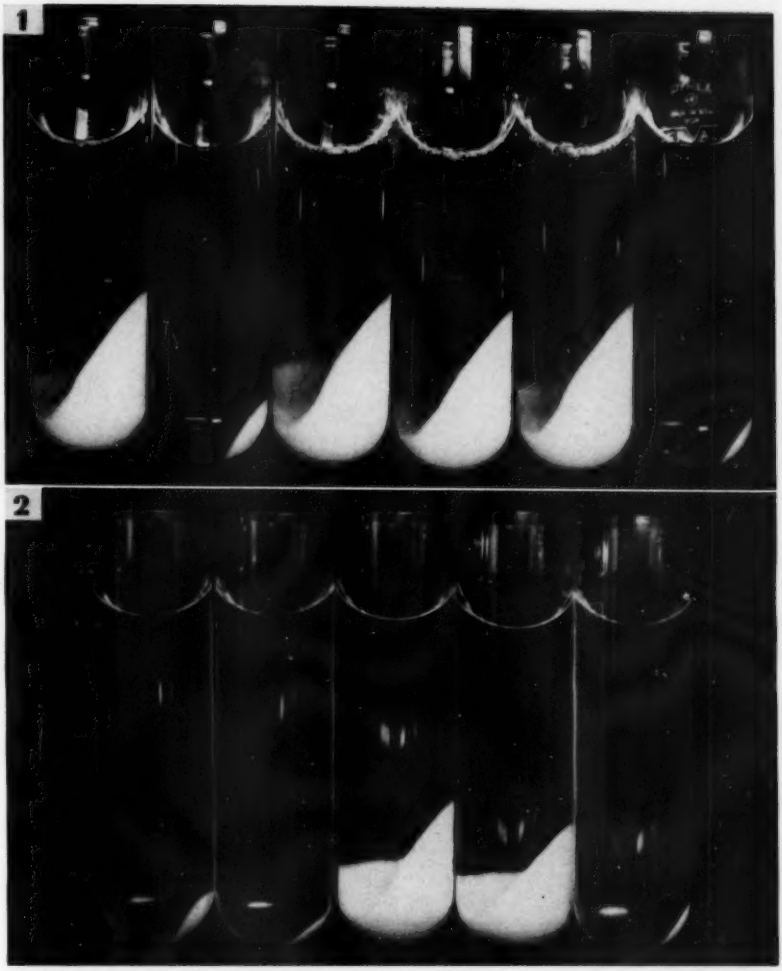

FIG. 1. Effect of thiamine-HCl, pyridoxine-HCl, Ca-pantothenate, and *d*-biotin on growth of *P. pullulans*. Cultures centrifuged at 3000 r.p.m. for 10 minutes. Left to right: complete mixture, less thiamine-HCl, less pyridoxine-HCl, less Ca-pantothenate, less *d*-biotin, control.

FIG. 2. Effect of thiazole and pyrimidine on growth of *P. pullulans*, cultures centrifuged as above. Left to right: thiazole, pyrimidine, thiazole and pyrimidine, thiamine-HCl, control.

Ca-pantothenate, and *d*-biotin, to that on media supplemented with yeast extract. As the synthetic medium alone supported only limited growth, determination of the vitamin requirements of the fungus was carried out by measuring growth when each of the vitamins was omitted in turn from the mixture. The results obtained are given in Table I and illustrated in Fig. 1; they demonstrate clearly the necessity of thiamine-HCl, the other three vitamins being without effect on growth.

TABLE I

The effect of the vitamins thiamine-HCl, pyridoxine-HCl Ca-pantothenate, and *d*-biotin on the growth of *P. pullulans**

Vitamin omitted	Yield (dry weight mg)
None	413.0 ± 9.4
Thiamine-HCl	46.4 ± 10.3
Ca-pantothenate	412.1 ± 6.2
Pyridoxine-HCl	409.7 ± 5.3
d-Biotin	409.7 ± 5.4
All	29.4 ± 1.7

*Inoculum 8.5×10^7 conidia per culture flask.

TABLE II

The effect of thiazole and pyrimidine on the growth of *P. pullulans**

Supplement	Yield (dry weight mg)
Thiazole	34.4 ± 2.5
Pyrimidine	39.6 ± 2.7
Thiazole and pyrimidine	407.3 ± 5.4
Thiamine-HCl	395.6 ± 7.6
Control	34.7 ± 0.5

*Inoculum 8.5×10^7 conidia per culture flask.

The thiamine requirement of many fungi is satisfied by either one of the two thiamine moieties, thiazole or pyrimidine, or by a mixture of the two (7). To determine the behavior of *P. pullulans* in this respect the synthetic medium was supplemented with either one or both of these two compounds and the amount of growth was compared with that which occurred in the presence of thiamine-HCl. The results are given in Table II and Fig. 2. Apparently the isolate of *P. pullulans* studied here is unable to synthesize either of the two thiamine moieties but can couple the two to form the thiamine molecule.

In view of the wide distribution of this fungus in nature it would be of interest to determine whether the thiamine requirement reported here is general for the species as a whole or is merely a strain characteristic.

Acknowledgments

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CELLULAR DEDIFFERENTIATION AND REDIFFERENTIATION IN SPLACHNUM AMPULLACEUM (L.) HEDW.[1]

MARY M. MACNUTT AND K. E. VON MALTZAHN

Abstract

Cellular dedifferentiation and redifferentiation are studied in leaves of the moss gametophore. Changes in the size and number of some cellular constituents as well as changes in their position are observed. Cell division, the plane of new wall formation, and factors affecting them are considered. Isolation of the leaf initiates events leading to the dedifferentiated state of the component cells. These changes include increase in the size of the nucleus and nucleolus and decrease in the size of the chloroplasts accompanied by an increase in chloroplast numbers. Changes in the position of cellular components are initiated by intravacuolar cytoplasmic layer formation leading to systrophe. Systrophe is considered to be an important event in dedifferentiation representing the final topography of the cellular constituents in the dedifferentiated cell. The distribution of clusters is observed, indicating loss of cellular polarity. In order for redifferentiation to take place, a new axis of polarity must be initiated. Redifferentiation leads ultimately to cell division. This division is characterized and the distribution of planes of new wall formation is shown.

Introduction

Differentiation leads to developmental determination. Determination is in most cases not an irreversible process. The stability of developmental determination is governed by correlations between various parts of the organism. Reversal of developmental determination may, therefore, be brought about by means of interference with the system of correlations governing determination, e.g. by means of isolation of component parts of the organism. Regeneration is an expression of reversal in determination. A study of some aspects of correlations inhibiting the realization of regenerative potentialities has recently been attempted for the present plant material (MacQuarrie and von Maltzahn 1959; von Maltzahn 1959; von Maltzahn and MacNutt 1958).

Regeneration takes place via cellular dedifferentiation and redifferentiation. In order to understand regeneration and the nature of correlations as they affect regeneration it was felt necessary to study some aspects of cellular dedifferentiation and redifferentiation and this is attempted in the present investigation.

Materials and Methods

The male strain of *Splachnum ampullaceum* (L.) Hedw. was used throughout the present study. The plants were grown in test tubes under sterile conditions on Beijerinck's inorganic nutrient solution solidified by the addition of 1% agar. The pH of the medium was adjusted to 6.5. The plants were grown under

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conditions of continuous light supplied from daylight fluorescent lamps and under a constant temperature of approximately 24° C. Gametophores used were always 20–21 days old. Cross sections of leaves were prepared in the following manner: the leaves were killed in acetic Zenker's fixative and, after sectioning at 14 μ, were stained in safranin and counterstained with fast green.

Results

The Leaf and Its Component Cells

Since it was shown in a previous study (von Maltzahn and MacNutt 1958) that considerable differences exist in the regenerative behavior of cells of leaves of different age, only leaves from the middle region of the gametophore were used in order to attain uniformity in age. Except for the midrib the leaf is only one cell thick. Leaf cell size and shape were measured here along a line parallel to the midrib and several rows of cells removed from it. Cell size is expressed in terms of cell surface area, cell shape in terms of cell length:width ratios. Only every second cell was measured and the results are represented in Figs. 1 and 2. They indicate some degree of differentiation between cells from the base to the tip of the leaf both in terms of cell size and cell shape. In addition to these morphological differences, some physiological differences were found between different cells of the same leaf. The tooth cells show a different staining behavior with fluorescent dyes compared to the other leaf cells. The tooth cells exhibit green fluorescence almost instantaneously when placed in a 1:10,000 acridine orange solution, while the other cells of attached whole leaves do not stain readily (see also Drawert 1938).

The Leaf Cell and Its Component Chloroplasts

In fully differentiated leaf cells little variation was found in the size of their component chloroplasts. A possible correlation between chloroplast size and cell size and chloroplast number and cell size may, however, exist. Figure 3 shows that differences in cell size are not reflected in the size of their component chloroplasts, while Fig. 4 indicates a close relationship between chloroplast number and cell size. The correlation coefficient is 0.79 indicating a highly significant linear relation. In order to exclude this variation in the number of chloroplasts due to cell size it was, therefore, necessary to compare cells from the same region of the leaf. The chloroplasts are irregular in shape and are packed closely together (Fig. 10). At the light intensities used, the chloroplasts are in peristrophe (Fig. 11) (Senn 1908).

Changes in Size and Number of Chloroplasts in Cells Undergoing Dedifferentiation

It had previously been shown that an isolated half leaf will regenerate, while a half leaf attached to a stem segment will not (MacQuarrie and von Maltzahn 1959). The behavior of cells of an attached half leaf seemed to parallel that of an attached whole leaf in terms of protonematal regeneration. Cells of the following systems were, therefore, compared: (*a*) an isolated half leaf, (*b*) a half leaf attached to a stem segment, and (*c*) a whole leaf attached to a stem segment.

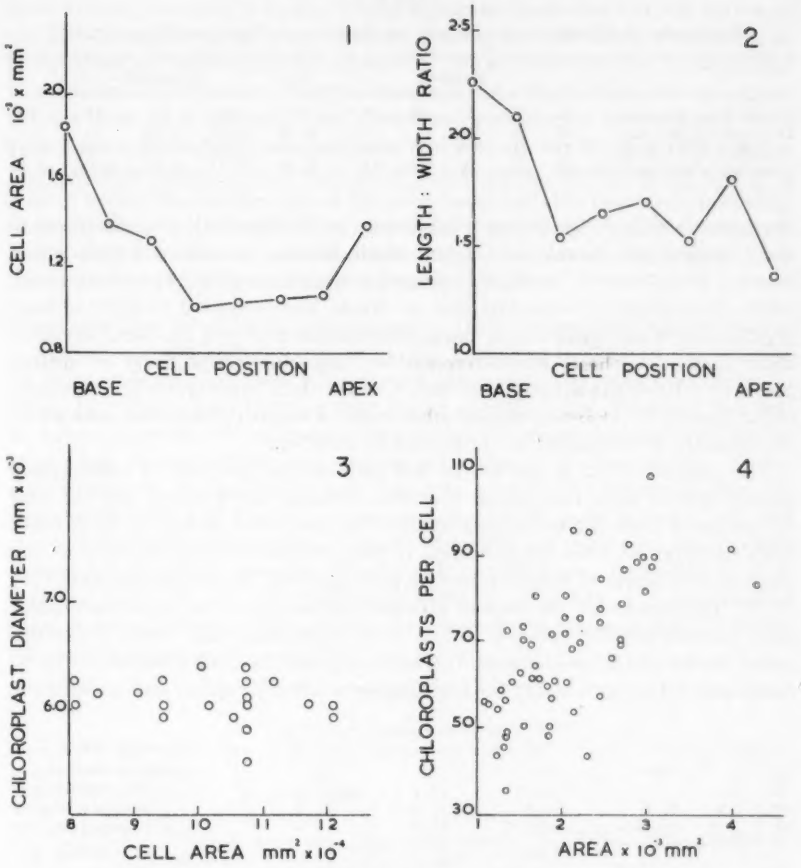

FIG. 1. Size of leaf cells in relation to their position in the leaf.
FIG. 2. Shape of leaf cells in relation to their position in the leaf.
FIG. 3. Relationship between chloroplast size and cell size in leaf cells.
FIG. 4. Relationship between chloroplast number and cell size in leaf cells.

The number of chloroplasts per cell was determined at selected time intervals after the preparations of the three systems. Observations were restricted to cells of the middle region of the leaf in order to minimize variations in chloroplast number due to size of cells. The results of a typical experiment are shown in Table I. Each figure represents an average of 30–40 cells with no more than four cells per leaf. Similarly chloroplast size was determined and these results are represented also in Table I. Each figure represents an average from 150 chloroplasts. A comparison between systems (*a*), (*b*), and (*c*) in Table I indicates that while chloroplast number increases greatly and chloroplast size

TABLE I

The number of chloroplasts per cell and the diameter of chloroplasts in mm × 10^{-3}

	Number					Diameter				
Hours after treatment	0	10	24	48	72	0	10	24	48	72
Isolated half leaf (*a*)	65	66	99	—	—	6.20	6.05	5.49	—	—
Attached half leaf (*b*)	73	69	75	75	79	6.03	6.08	6.13	6.06	6.11
Attached whole leaf (*c*)	69	68	78	74	72	6.05	5.97	6.01	6.06	6.04

decreases in cells of the isolated half leaves, such changes do not take place in the attached half leaves or attached whole leaves. In cells of detached half leaves a prevalence of "dumbbell"-shaped chloroplasts (Fig. 14) was observed, while chloroplasts of attached half or whole leaves tended to have a more regular oval or spherical shape. Since chloroplasts multiply by direct division, these shape differences emphasize further the divisional activity of chloroplasts in cells of the isolated half leaf. Cellular dedifferentiation here brought about simply by isolation results apparently in an increase in the number of chloroplasts accompanied by a decrease in their size.

The possible effect of age of the leaf cells on the behavior of chloroplasts during cellular dedifferentiation was also investigated. Systems (*a*), (*b*), and (*c*) prepared from leaves located toward the apex were compared with these systems obtained from basal leaves. It had previously been shown that the regenerative power of leaves decreases with age (von Maltzahn and MacNutt 1958). The results for the present observations are given in Fig. 6 for chloroplast number per leaf cell and in Fig. 5 for chloroplast size, where A denotes apical leaves and B basal leaves. It should be noted that the number of chloroplasts per cell is much less in the basal leaves while chloroplast size is about the

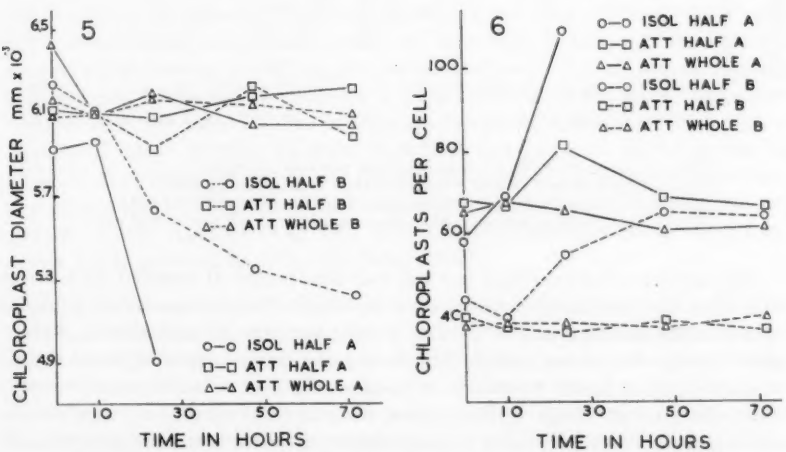

FIG. 5. Changes in chloroplast size in cells of apical (A) and basal (B) leaves.
FIG. 6. Changes in chloroplast number in cells of apical (A) and basal (B) leaves.

same in the two types of leaves. These figures indicate that like the leaves of the middle region of the gametophore, both apical and basal detached half leaves show an increase in the number of chloroplasts per cell accompanied by a decrease in their size. The attached half and attached whole leaves again did not show these changes. The differences between cells of apical and basal leaves are expressed in the magnitude and rate of the changes. The chloroplast number in the cells of the apical leaf increases more quickly and to a greater extent than in the cells of the older basal leaf. Decreases in chloroplast diameter occurred in both apical and basal leaves which had been isolated, but as with the increase in chloroplast number, the older leaves reacted more slowly and to a lesser extent than the younger leaf.

Changes in the Size of Nuclei and Nucleoli in Cells Undergoing Dedifferentiation

Nuclear size was also determined in cells belonging to systems (*a*), (*b*), and (*c*) at selected time intervals following the treatment. The results are shown in Table II. These data indicate that nuclei of cells of the detached half leaf undergo a considerable increase in size while cells of the attached half leaf or whole leaf do not exhibit any significant changes in nuclear size. Direct observations on nucleolar size were found to be difficult. However, prepared slides of isolated half leaves in cross section stained with safranin and fast green yielded some information. Figures 11 and 16 are camera lucida drawings showing nuclear and nucleolar size in cells of half leaves immediately after isolation (Fig. 11) and 48 hours after isolation (Fig. 16). A comparison of these figures indicates that nuclear size increase is accompanied by a considerable increase in nucleolar size.

TABLE II

The diameter of the nucleus in mm $\times 10^{-3}$

Hours after treatment	0	10	24	48	72
Isolated half leaf	6.14	6.52	7.50	—	—
Attached half leaf	6.39	6.14	6.68	6.29	6.74
Attached whole leaf	6.42	6.23	6.77	6.70	6.66

Cellular dedifferentiation here brought about simply by isolation results clearly in an increase in nuclear and nucleolar size indicating renewed synthetic activities of the protoplast of the dedifferentiating cell.

Changes in the Position of the Cytoplasm, Nucleus, and Chloroplasts in Dedifferentiating Cells

In a mature leaf cell the cytoplasm is restricted to a thin layer inside the cell wall surrounding a large vacuole (Fig. 11). Figure 11 shows also the relative position of the nucleus and the chloroplasts inside the cytoplasm in cross-sectional view; Fig. 10, that of the chloroplasts in surface view. In the present observations on the configuration of some main protoplasmic components cells of a detached half leaf were compared with those of an attached half leaf.

While a protoplasmic configuration characteristic for mature leaf cells is maintained in cells of the attached half leaf and whole leaf, conspicuous changes

in protoplasmic configuration take place in cells of the isolated half leaf. Within a few hours after isolation a primary cytoplasmic layer is formed across the vacuole connecting directly the cytoplasm around the nucleus with that on the opposite side of the vacuole (Fig. 12). Following this primary layer formation, other intravacuolar cytoplasmic layers are formed from other parts of the peripheral cytoplasm. This is shown in Fig. 13, in which the chloroplasts have been omitted in order to simplify the drawing. The nucleus moves via the primary cytoplasmic layer to a more central position in the cell (Figs. 15 and 16). The cytoplasmic layers similarly facilitate a migration of the chloroplasts from their peripheral to a central position leading to the formation of a tight cluster of chloroplasts around the nucleus (Figs. 15 and 16). This formation of a cluster of chloroplasts is known as systrophe (Senn 1908); the chloroplasts change, therefore, in the present case from a position of peristrophe to a position of systrophe. Since the nucleus in the present material is always found in the center of the chloroplasts and since the migration of the nucleus appears to precede chloroplast migration, the position of the chloroplasts in systrophe seems to be determined by the position of the nucleus, cytoplasmic migration being, however, the primary event.

The Position of the Clusters and Factors Affecting It

In order to study the relative position of the clusters in cells of the isolated half leaf, isolated half leaves were examined 48 hours after isolation. The cells examined did not include the three to four rows of cells next to the wound surface in order to exclude possible wound influences on cluster position. The position of the clusters inside the cells was examined in surface plane only. For convenience in tabulating the results, the cell was divided into five sections as shown in Fig. 9. The surface area of the cell was, for purposes of discussion, divided into rectangles of arbitrary but about equal size with two sides parallel to the longitudinal axis of the leaf. Part A contained all those clusters which were oriented toward the tip of the leaf, part B those toward the base, part C those with the clusters in the center of the cell surface area, while parts D and E showed orientation toward the outer leaf edge and toward the midrib respectively. The relative frequency of cluster position in the various parts is shown in Table III. Since the clusters occur at about the same frequency in

TABLE III

The orientation of clusters in leaf cells

	Number of observations		
		Cells near wound surface	
Region of cell	Cells not near wound surface	Wound surface at right angle to midrib	Wound surface parallel to midrib
A	41	6	3
B	36	42	1
C	41	7	1
D	35	9	53
E	38	12	3

each section, it may be concluded that in the cells not in the neighborhood of the wound the clusters show random distribution.

The effect of wounding on the orientation of the clusters was also investigated. The position of the clusters was observed in those three to four rows of cells at the wound surface at the base of the leaf. The results, shown in Table III, indicate non-random distribution of the clusters in the cells near the wound surface, the clusters exhibiting an orientation toward the wound surface.

Leaves were also isolated at their base and a wound inflicted parallel to the midrib by means of cutting off the outer edge of the leaf. Cells near the wound surface were here examined again and the results (Table III) show that the clusters are oriented toward the wound surface in cells near the wound regardless of the relation of the wound surface to the longitudinal axis of the leaf.

Cell Division and Cellular Redifferentiation

Random distribution of the clusters in dedifferentiated cells suggests that the process of cellular dedifferentiation results in the loss of cellular polarity. Under the present experimental conditions dedifferentiation is followed ultimately by the formation of a regenerative protonema which represents a polar structure. The initiation of a protonema is preceded by karyokinesis and cytokinesis of the dedifferentiated cell. It has already been stated that the nucleus is present in the center of the cluster. During the period 48–60 hours after leaf isolation, the nucleus divides and cell wall formation takes place along the most prominent intravacuolar cytoplasmic layer, this layer representing, therefore, the phragmosome. This is followed by the gradual disappearance of the remaining cytoplasmic layers.

In order to investigate the plane of the new cell wall, formed as a result of cytokinesis, in relation to the polar axis of the leaf, leaves from the middle region of the gametophore were isolated. Seventy-two hours after isolation of the leaf, measurements were made of the angle between the newly formed cell wall and the longitudinal axis of the leaf in surface view by means of a goniometer eyepiece. The results for the cells of an isolated leaf excluding the cells near the wound surface are shown in form of a histogram in Fig. 7. From this figure it may be seen that the angles fall into two separate classes. The two extreme values, i.e., angles of 0–9° and 170–179°, represent 20% of the total population. In this class the plane of the new cell wall in surface view is nearly parallel to the polar axis of the leaf. The mean value for the remaining angles is 88°. This represents a right angle to the polar axis of the leaf. Hence, there is a tendency in cells of the normal leaf for the new cell wall to be formed either perpendicular or parallel to the polar axis of the leaf.

In order to study a possible effect of the wound surface on the plane of new cell wall formation in cells near the wound surface, a wound was inflicted parallel to the midrib and the angle between the plane of the new cell wall and the polar axis of the leaf was measured in cells near the wound surface 72 hours after isolation. These data are also represented in the form of a histogram as shown in Fig. 8. In these cells the new cell wall tends to be approximately parallel to the wound surface.

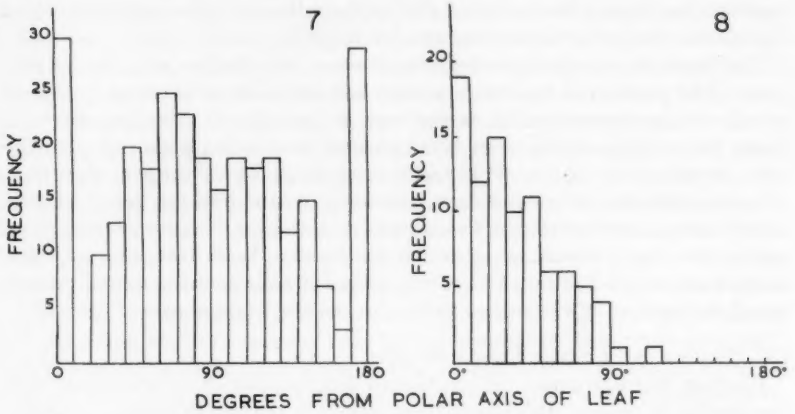

FIG. 7. Histogram representing the relative frequency of angles formed by the new cell wall with the polar axis of the leaf in surface view (for leaf cells not in the vicinity of the wound surface).

FIG. 8. Histogram representing the relative frequency of angles formed by the new cell wall with the polar axis of the leaf in surface view (for leaf cells in the vicinity of the wound surface).

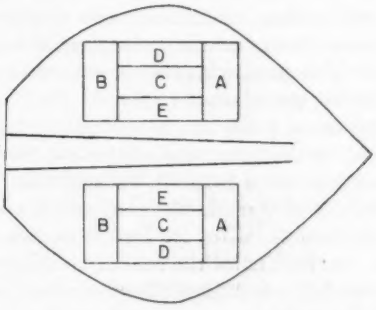

FIG. 9. Diagram of leaf, rectangles representing cells divided into five parts (A, B, C, D, and E).

Cytokinesis results in the formation of two daughter cells, one daughter cell representing the protonematal initial, the other daughter cell the "remaining cell". The protonematal initial can be recognized shortly after nuclear division of the mother cell, since it forms a protuberance, the protonema. Cell wall formation divides the chloroplasts into two lots which move via the remaining cytoplasmic layers toward the periphery of the newly formed daughter cells. Figure 17 shows a surface view of a cell 48–60 hours after isolation in which new cell wall formation has already been completed. This figure indicates an equal distribution of the chloroplasts to the two daughter cells. The number of chloroplasts present in the remaining cell appears to be the same as that in the mother cell before dedifferentiation. In one set of cells.

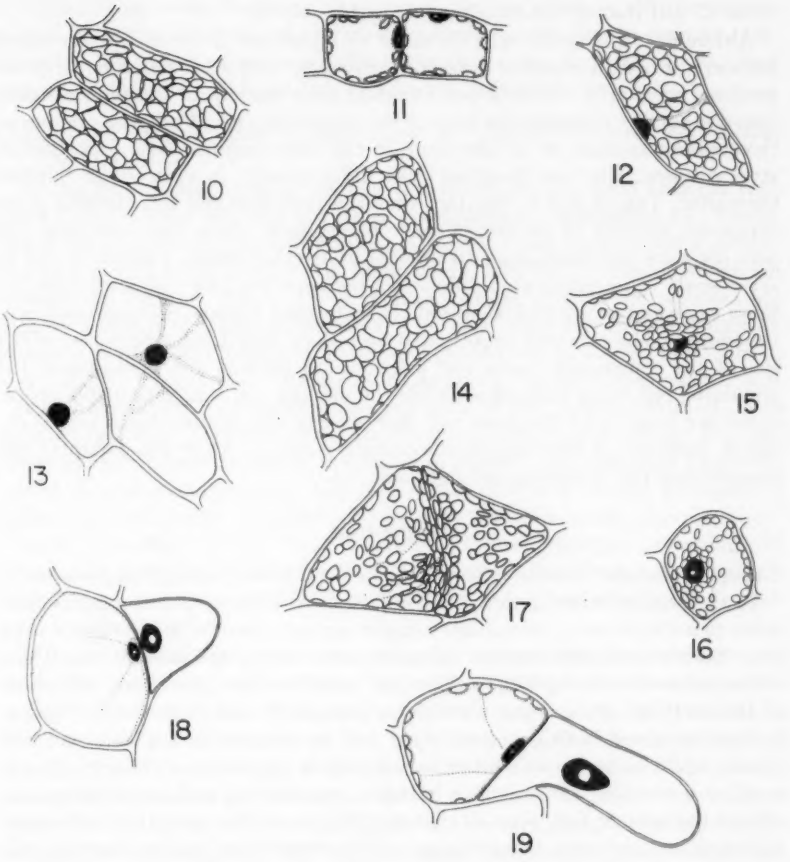

FIG. 10. Surface view of leaf cells immediately after leaf isolation.
FIG. 11. Cross-sectional view of leaf cells immediately after leaf isolation.
FIG. 12. Surface view of leaf cell 6 hours after leaf isolation, showing primary cytoplasmic layer formation.
FIG. 13. Surface view of leaf cells 10 hours after leaf isolation. Chloroplasts have been omitted from drawing.
FIG. 14. Surface view of leaf cells 12 hours after leaf isolation, showing dumbbell-shaped chloroplasts.
FIG. 15. Surface view of leaf cell 48 hours after leaf isolation.
FIG. 16. Cross-sectional view of leaf cell 48 hours after leaf isolation.
FIG. 17. Surface view of leaf cell 48–60 hours after leaf isolation.
FIG. 18. Cross-sectional view of protonematal initial and remaining cell shortly after formation of protonematal protuberance. Chloroplasts have been omitted from drawing.
FIG. 19. Cross-sectional view of protonematal initial and remaining cell. Chloroplasts have been omitted from drawing of protonema.

the number of chloroplasts of the mother cell at the time of isolation was found to be 87 and that of the remaining daughter cell 94.

Although cell division appears to be an equal one in terms of chloroplast numbers, it is an unequal or differential division in all other respects. In cross-sectional view (Fig. 18) it is quite evident that the size of the protonematal initial is smaller compared to that of the remaining cell. While the number of chloroplasts appears to be the same in the two daughter cells immediately after division, the two daughter cells differ greatly in chloroplast number thereafter. This is due to the fact that the protonematal cell exhibits great divisional activity of its component chloroplasts, while the remaining cell does not show any increase in the number of its chloroplasts. Figures 18 and 19 show some other important differences between the two daughter cells. In these cases the protonematal initials have formed already protonematal protuberances. The two cells represented here show additional differences in the size of the component nuclei and nucleoli, the nucleus and nucleolus of the remaining cell being quite small while the nucleus and nucleolus of the protonema are large. The daughter cell representing the protonematal initial exhibits, therefore, a high degree of synthetic activity, while the daughter cell representing the remaining cell does not.

Discussion

Change in Size and Number of Some Cellular Components during Dedifferentiation

In the present investigation it is shown that isolation of gametophore leaves leads to an increase in chloroplast number accompanied by a decrease in their size. Nuclear and nucleolar size also increase in cells of the isolated leaf. These observations on chloroplast, nuclear, and nucleolar behavior agree with those of Heitz (1925). This author showed for *Lophocolea* and *Funaria* that nuclear volume increased both in regenerating and non-regenerating cells of isolated leaves, while nucleolar volume increased only in regenerating cells. In *Splachnum* no distinction can be made between regenerating and non-regenerating cells of the isolated leaf, since all cells appear to have the capacity to regenerate following leaf isolation. Luise Stange (1959) observed in dedifferentiating cells of *Riella* an increase in nucleolar size, dedifferentiation having been initiated in her material by isolation from the apical meristem. A comparison between cells of attached and detached half leaves indicates clearly that increase in nuclear and nucleolar volume is the result of isolation, also in the present material. Schoser (1956) investigated regeneration in the Cladophoraceae and found an increase in the number of nuclear divisions at the basal pole of the multinucleate cell following cellular isolation. This was accompanied by an increase in nuclear size. The DNA content of the nucleus of the regenerating cell showed an increase over that of the resting cell. While this question was not investigated in the present material, it most likely occurs here too. Stich (1951) reported for *Acetabularia mediterranea* that there is a close correlation between nuclear and nucleolar size on the one hand and cytoplasmic synthesis on the other. Caspersson and Schultz (1940) have indicated that there is an

intimate association between the nucleolus, ribonucleoproteins, and protein synthesis. They state that any activity in the nucleolus is always accompanied by active synthesis of cytoplasmic nucleic acids. In the present case, following leaf isolation the nucleolus showed a rapid increase in size, thus suggesting that the cell is actively engaged in protein synthesis.

Changes in Intracellular Configuration of Cellular Components during Differentiation

In dedifferentiating cells of the leaf of *Splachnum*, profound changes take place in the relative position of various protoplasmic components, i.e., formation of intravacuolar cytoplasmic layers, migration of the nucleus from a peripheral to a more central position via these layers, followed by cluster formation around the nucleus. Heitz (1925) reported similar events for regenerating cells of leaves of *Lophocolea bidentata*. The most thorough investigations on phenomena of cluster formation have been carried out by Germ (1931, 1933*a*, and 1933*b*). This author distinguishes between "plasma systrophe" and "plastid systrophe". In dedifferentiating cells of the present material, both types of systrophe take place together. This is apparently not always the case. Germ (1933*b*) observed plastid systrophe in mosses after they had been subjected to intense illumination. Günther (1957) described plastid systrophe in cells of isolated leaves of *Elodea*. These cells died 3–4 weeks after isolation. Sinnott and Bloch (1941) have described plasma systrophe preceding cell division in vacuolate cells in normal histogenesis. The present observations suggest that systrophe must be considered to be an important event in cellular dedifferentiation.

Various explanations have been proposed to account for phenomena of systrophe. In Germ's work (1932, 1933*a*, and 1933*b*) systrophe was mostly brought about by means of plasmolysis and he considers it to be a reaction of living protoplasm to plasmolysis, i.e., the irritation caused by the removal of the protoplast from the cell wall. Cellular dedifferentiation accompanied by systrophe in cells near a wound surface is considered by Sinnott and Bloch (1941) to be the result of wounding. In the present material cellular dedifferentiation accompanied by intravacuolar cytoplasmic layer formation, plasma, and plastid systrophe results simply from isolation. This supports Heitz's original observations and is in agreement with Bünning (1955). Germ's results can be interpreted in the same way, plasmolysis acting as a means of isolation. The withdrawal of the protoplast from the cell wall with subsequent disruption of protoplasmic connections between cells removes each cell from the correlative influence of its neighbors. Thus the phenomena observed by Germ and brought about by plasmolysis may also represent part of the process of cellular dedifferentiation.

In surface view the clusters showed random distribution in cells not in the neighborhood of the wound surface. In cross-sectional view the position of the clusters tended to be near the center of the cell. Random distribution of clusters may be considered to be the result of loss of cellular polarity, this loss being

the result of cellular dedifferentiation. While isolation and not wounding initiates cellular dedifferentiation, wounding does have an effect on the position of clusters in cells near the wound surface, the clusters being oriented here toward the wound. Nestler (1898) described positive traumatotactic movement of the nucleus and cytoplasm toward a wound surface. Magda Ziegler (1955) showed that the positive traumatotactic movement of the nucleus is preceded by that of the cytoplasm. This latter movement followed by that of the nucleus would appear to determine the orientation of clusters toward the wound surface. Magda Ziegler showed furthermore that the wound stimulus reaction resulted in reversible volume changes in the nucleus and an increase in nucleolar size indicating renewed cytoplasmic synthesis.

Cellular Redifferentiation and Cell Division

It has been stated earlier in the present discussion that cellular dedifferentiation results apparently in loss of cellular polarity. Following redifferentiation, the regenerating cell ultimately undergoes cell division and this division represents an unequal or differential division. In order for this division to take place, a new axis of polarity must be established. Initiation of a new axis of polarity must take place during the process of cellular redifferentiation. It may be assumed that the axis of the mitotic spindle apparatus in its final position will be parallel to or coincide with the new axis of polarity. Since the plane of the new cell plate will be at a right angle to the axis of the spindle, one may be justified in considering the position of the newly formed cell wall as an indicator for the position of the new axis of polarity. The line formed by the fusion of the new cell wall with the upper surface wall shows in surface view a tendency to be either parallel or perpendicular to the longitudinal axis of the leaf in cells not in the vicinity of the wound surface. One of the preferred orientations for the new axis of polarity is, therefore, parallel to the diagonal of the nearly isodiametric cell as seen in cross-sectional view. The other preferred orientation for the new axis of polarity is parallel to the diagonal of the nearly rectangular cell as seen in longitudinal-sectional view. It cannot be decided at present whether geometrical factors, light gradients, or chemical gradients determine the induction of a new axis of polarity and its orientation; a more thorough investigation of this question will be necessary. Cells in the neighborhood of the wound surface show an orientation of the newly formed cell walls parallel to the wound surface as seen in surface view irrespective of the relative orientation of the wound surface in relation to the axis of the leaf. Similar relationships between the orientation of the cell wall and that of the wound surface have been described for other plant material (Sinnott and Bloch 1941). Hence in cells near the wound surface, gradients in wound substances must be the predominant factor determining the orientation of the new axis of polarity as expressed by the position of the new cell wall.

Cell division leads to the formation of the protonematal initial and the remaining cell. Distribution of chloroplasts to the two daughter cells appears initially to be equal in the present case. Unequality in divisional activity of the chloroplasts in the two daughter cells results in considerable differences in the

number of chloroplasts between the two daughter cells, the protonematal cell containing a large number while the number of chloroplasts stays small in the remaining cell. Zepf (1952) has described unequal division in the developing leaf of *Sphagnum* leading to differentiation between an active chlorophyll-bearing cell and the hyaline cell which is nonliving at maturity. The distribution of chloroplasts to these two daughter cells is here also equal initially while their subsequent fate is quite different.

In the present material cell division is an unequal one in all other respects, the protonematal initial being much smaller, having denser cytoplasm and containing a much larger nucleus and nucleolus compared to the remaining cell. These observations indicate that the protonematal initial exhibits great synthetic activities while the remaining cell does not. The possibility is, however, not excluded that the remaining cell may still be reactivated when isolated as is the case with hyaline cells during earlier stages of development (Zepf 1952).

Magda Ziegler (1955) showed for epidermal cells of *Allium cepa* that the wound stimulus brought about a displacement of the cytoplasm toward the wound surface in cells near the wound surface. Geitler (1955) found in a study of normal pollen development in *Gasteria cheilophylla* differential divisions similar to those described here for the present material. He (Geitler 1935) suggested that the displacement of cytoplasm is the most important factor and questioned whether there was initially any new synthesis of cytoplasm. If nucleolar size is taken as an indicator of new synthesis of cytoplasm, it would appear in the present case that both displacement and new synthesis are involved.

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CULTURAL STUDIES OF FLAMMULA ALNICOLA (FR.) KUMMER AND FLAMMULA CONISSANS (FR.) GILLET[1]

W. B. G. DENYER[2]

Abstract

A fungus isolated from a yellow, stringy decay of white spruce and other conifers in Western Canada was at first identified as *Flammula conissans* sensu Ricken. This fungus was compared with an authentic isolate of *Flammula conissans* (Fr.) Gillet obtained from Europe. They were shown to be separate species through comparison of cultures, fruit bodies, spores, and the factors affecting fruiting in culture, as well as by pairings of monosporous mycelia. The Canadian fungus was identified as *Flammula alnicola* (Fr.) Kummer. Basidiospores of *F. alnicola* germinated after cold treatment at −7° C. The requirement of moderate cold-treatment for spore germination may account for the occurrence of the fungus only as a root-rot pathogen.

Introduction

A fungus, known only as "hemlock agaric" prior to 1950, was first isolated from a yellow, stringy decay in western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in the Queen Charlotte Islands and later from white spruce (*Picea glauca* (Moench) Voss and *P. glauca* var. *albertiana* (S. Brown) Sarg.) and other conifers in Western Canada. It was called the "hemlock agaric" fungus because agaric-like primordia developed in old cultures. In 1950 an agaric associated with the yellow stringy "hemlock agaric" decay in *P. glauca* var. *albertiana* was collected by Dr. C. G. Riley and me, at Seebe, Alberta. Cultures from the context of the agaric were identical with "hemlock agaric" cultures isolated from decay. On the basis of this collection the fungus was identified tentatively as *Flammula conissans* sensu Ricken and American authors. This paper reports on studies of the fungus in comparison with an authentic isolate of *Flammula conissans* (Fr.) Gillet, obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, which showed them to be separate species. The Canadian isolate was identified by Dr. J. W. Groves[3] as *Flammula alnicola* (Fr.) Kummer.

Descriptions of Cultures

The following descriptions of cultural characters conform in method and style with those of other species of wood-rotting basidiomycetes published by Nobles (11). Cultures of the isolates of *F. alnicola* studied by the author correspond with the following description, which was supplied by Dr. Nobles.[3]

[1]Manuscript received June 28, 1960.
Contribution No. 664, Forest Biology Division, Research Branch, Canada Agriculture, Ottawa, and No. 60, Research Station, Canada Agriculture, Saskatoon, Saskatchewan; part of a thesis submitted to the University of British Columbia in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
[2]Forest Biology Laboratory, Saskatoon, Saskatchewan.
[3]Plant Research Institute, Research Branch, Canada Agriculture, Ottawa.

Flammula alnicola (Fr.) Kummer

KEY PATTERN: (1,2) 1 1 1 (7,9) 2 2 2 4 2 2

CULTURES EXAMINED:

CANADA.—British Columbia: Queen Charlotte Islands, on *Tsuga heterophylla*, DAOM17168. Alberta: Seebe, on *Picea glauca* var. *albertiana*, DAOM25874, and other isolates from *P. glauca*.

CULTURAL CHARACTERS:

GROWTH CHARACTERS.—Growth very slow, radius up to 6.5 cm in 6 weeks (Fig. 1). Advancing zone even, slightly raised, with aerial mycelium to limit of growth. Mat white at first and remaining so or becoming pale yellow (Munsell (10) color equivalent: 2.5Y 8/6) after 2 to 3 weeks, slightly raised, fine woolly, aggregated in small tufts so arranged as to give a zonate or radiate appearance in some cultures, occasionally with compact yellow balls (abortive fruit body primordia) usually appressed to the wall of the petri dish. Reverse unchanged or pale yellow. Odor penetrating, musty. On gallic and tannic acid agars, diffusion zone very weak to moderately strong; no growth on either medium. Gum guaiac test (13) positive.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, branched at and between septa, 1.5–3.1 μ diameter. *Aerial mycelium*: hyphae as in the advancing zone, occasionally with golden yellow contents; allocysts[4] (Fig. 3) very numerous, usually terminal (formed singly or in chains of two or three), occasionally intercalary, thin-walled, at first hyaline with granular or oily contents deeply staining in phloxine, later rarely yellow, more frequently empty, subglobose to ovoid or pyriform, 7.5–15.6 × 7.5–23.5 μ. *Submerged mycelium*: hyphae and allocysts as in aerial mycelium. Allocysts numerous in monosporous cultures.

TYPE OF ROT: Yellow stringy root and butt rot with laminate and radiate pattern in cross section. The fungus has been isolated and identified from decay in *Tsuga heterophylla*, *Abies balsamea* (L.) Mill., *A. amabilis* (Dougl.) Forb., *A. lasiocarpa* (Hook.) Nutt., *Picea glauca*, *P. glauca* var. *albertiana*, *P. mariana* (Mill.) B.S.P., *P. engelmannii* Parry, *Pinus banksiana* Lamb., *P. contorta* Dougl. var. *latifolia* Engelm., *Betula lutea* Michx. f., and *Populus* sp., from British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick, and Nova Scotia.[5]

Flammula conissans (Fr.) Gillet

KEY PATTERN: 1 1 1 1 (7,9) 2 2 2 2 2 2

CULTURES EXAMINED:

DAOM22888 from Baarn, Holland, isolated by Kühner, but locality and host not known.

CULTURAL CHARACTERS:

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in 3 to 4 weeks (Fig. 2). Advancing zone white, even, raised, 0.5 cm wide, with aerial mycelium to limit of growth. Mat white at first and remaining so, or with patches of pale yellow (2.5Y 8/4, 2.5Y 8/6), felty. Reverse unchanged. No odor. Reaction with gum guaiac positive.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, branched at and between septa, 1.5–5.0 μ diameter. *Aerial mycelium*: hyphae as in advancing zone; allocysts (Fig. 3) very numerous, terminal, occasionally intercalary, mostly solitary, occasionally in chains of two or three, at first hyaline with granular or oily contents deeply staining in

[4]See Snell, W. H. and Dick, W. A. 1957. A glossary of mycology. Harvard University Press, Cambridge, Massachusetts. p. 5.

[5]This host list, apart from collections made in the Prairie Provinces, has been compiled from collections made by the staffs of the Forest Biology Laboratories across Canada.

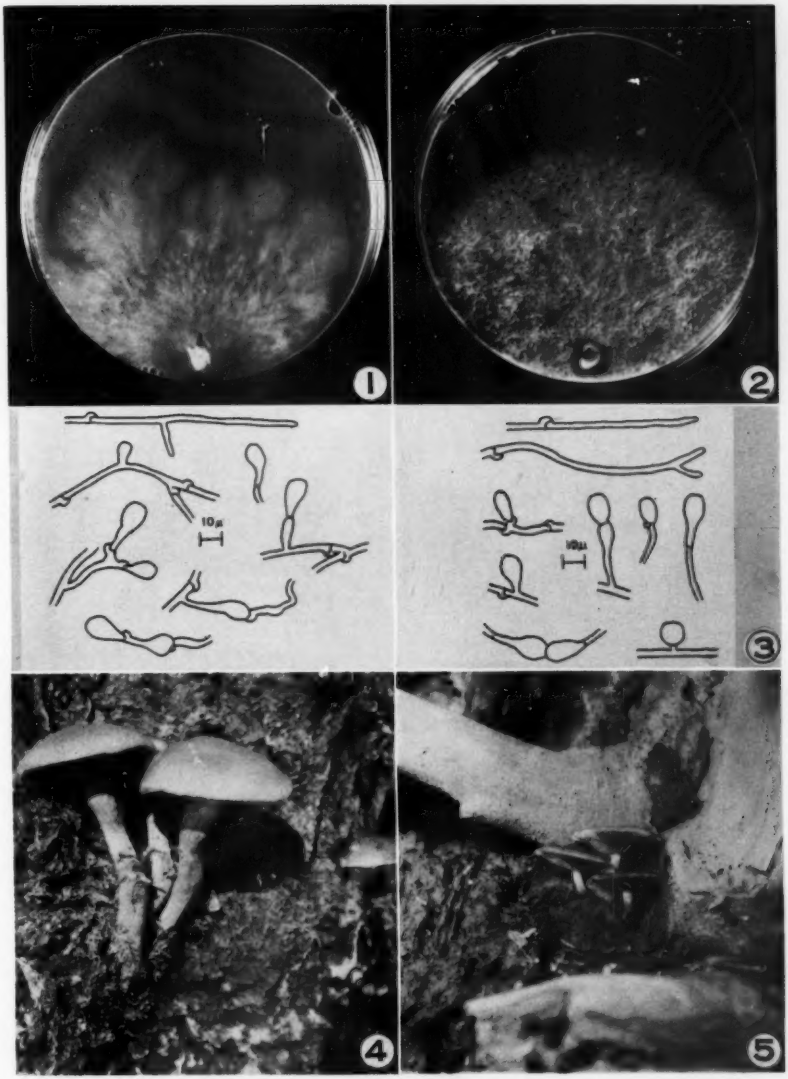

FIG. 1. *F. alnicola*, DAOM25874, on malt agar, 6 weeks old.
FIG. 2. *F. conissans*, DAOM22888, on malt agar, 3 weeks old.
FIG. 3. Hyphae from advancing zone, and allocysts of *F. alnicola* (left), and *F. conissans* (right).
FIG. 4. *F. alnicola* fruiting in the duff at the base of a white spruce stump. The duff covering the base of the mushrooms has been removed.
FIG. 5. *F. alnicola* fruiting on the roots of a white spruce stump partially excavated the preceding year.

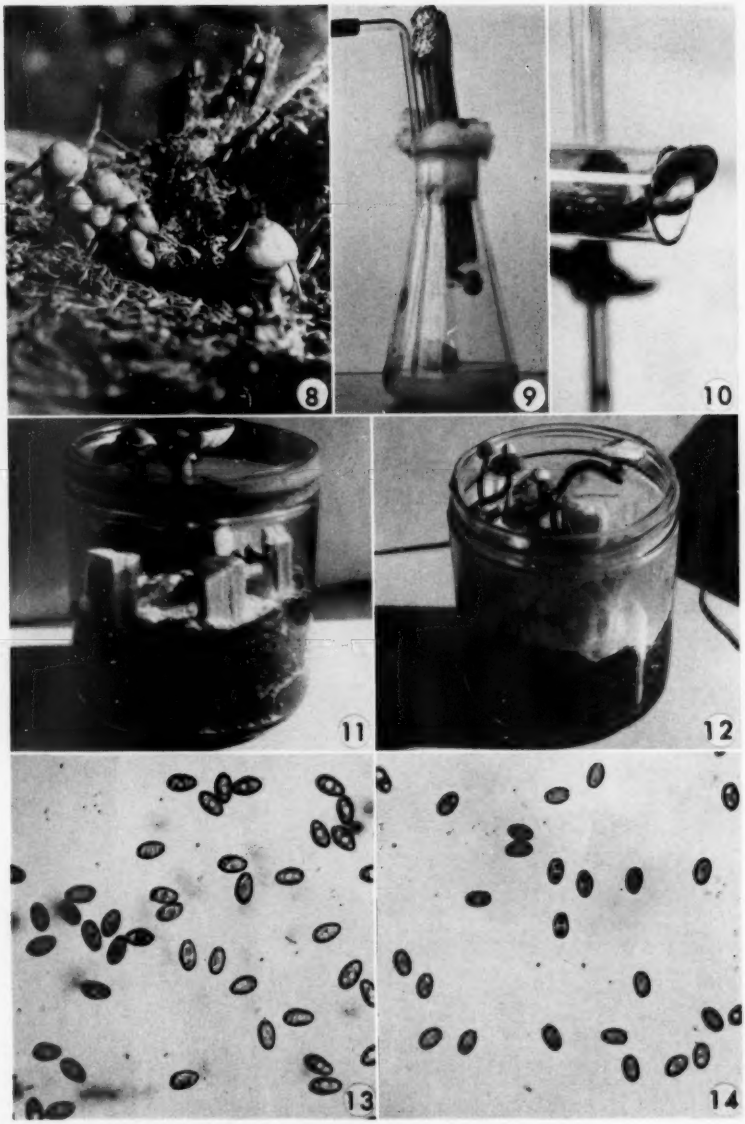

FIG. 8. *F. alnicola* fruiting on the litter-covered surface of a white spruce stump.
FIG. 9. *F. alnicola* fruiting in a Badcock fructification flask. Humidified air was introduced to the flask via the tube.
FIG. 10. Detail of fruit body in Fig. 9.
FIG. 11. *F. alnicola* fruiting on a soil and wood-block medium.
FIG. 12. *F. conissans* fruiting on a soil and wood-block medium.
FIG. 13. Basidiospores of *F. alnicola*. ×720
FIG. 14. Basidiospores of *F. conissans*. ×720

phloxine, later rarely yellow, more frequently empty, subglobose to ovoid or pyriform, 6.3–8.8 × 11.3–20.0 μ. *Submerged mycelium*: hyphae and allocysts as in aerial mycelium. Allocysts usually numerous in monosporous cultures.

TYPE OF ROT: *F. conissans* has not been isolated from decay in Canada. A yellow, stringy decay was produced in roots of *Picea glauca* inoculated with the fungus. Lange (9) and Kühner and Romagnesi (8) reported the fruit bodies on *Salix cinerea* L.

Allocysts are a characteristic feature of cultures of both species of *Flammula*; they do not differ enough in size and shape to be a basis for separation. Cultures of the two species may be separated readily by the characteristic musty odor of *F. alnicola* (lacking in *F. conissans*), the more rapid growth of *F. conissans*, and the appearance of the culture mat. Cultures of *F. alnicola* can usually be identified by their odor alone. Rarely an isolate may be odorless, but the appearance of the mat and the presence of allocysts identify this species.

The effect of pH on the growth of the two fungi on Difco malt agar buffered with McIlvaine's buffer (2) is shown in Fig. 6. *F. alnicola* grew faster and is apparently more tolerant of pH below 4.4 than *F. conissans*. *F. conissans* grew faster above pH 4.4. The buffer apparently had an adverse effect on growth apart from pH, since *F. alnicola* grew more rapidly on unbuffered malt agar (pH 5.5) than on malt agar buffered to pH 5.4.

Optimum temperature for growth of *F. alnicola* on Difco malt agar was 17° to 20° C. Etheridge (5) reported that *F. alnicola* would not grow at 30° C.

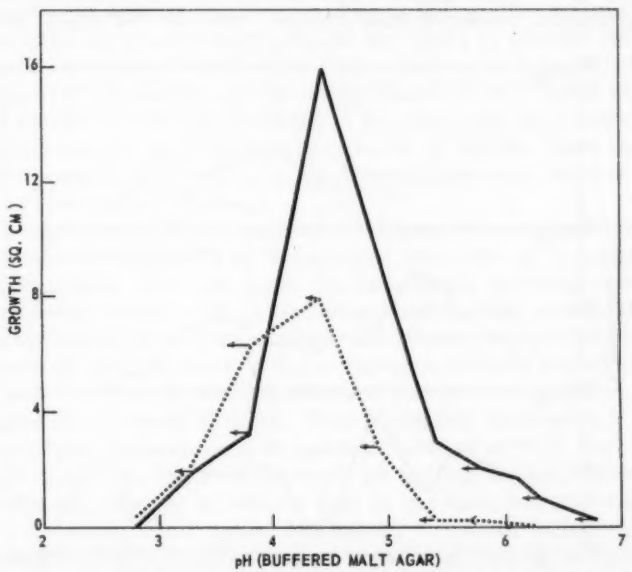

FIG. 6. The growth of *F. alnicola* (broken line) and *F. conissans* (solid line) in 4 weeks on malt agar buffered with McIlvaine's standard buffer solution. The arrows indicate, quantitatively, the change in pH of the medium during the test.

Fruiting of *F. alnicola* in Nature

Observations on the fruiting habit of *Flammula alnicola* were made in the Smith – Slave Lake region of Alberta. The mushrooms usually develop on dead trees or stumps, and rarely on living trees. They occur most commonly on infected wood that has been torn apart or otherwise exposed, such as the surface of stumps, and butts and roots that have been broken or exposed by windfall or road-building machinery. Occasionally they are found in the duff with bases appressed to the bark of stumps (Fig. 4). They are always associated with decayed wood of the host tree.

During a 5-year period fruit bodies were collected from August 2 to September 22, but mostly in the second half of August. In one instance fruit bodies were collected twice from one location on August 2 and September 8. Fruit bodies collected in September were usually found in protected locations on roots (Fig. 5).

The relation of sporophore development to temperature and relative humidity was investigated. Hygrothermograph records for 1954 and 1955, and thermograph records for 1956, were obtained for the summer months in the immediate vicinity of a white spruce stump containing *F. alnicola* decay. The record was started 3 weeks after the tree was felled, in 1954.

A five-point moving average of daily mean temperature (calculated by averaging the temperature at 3-hour intervals) for the three summers is shown in Fig. 7. No sporophores developed in the recorded period, but sporophore primordia developed from decayed wood on the stump surface in

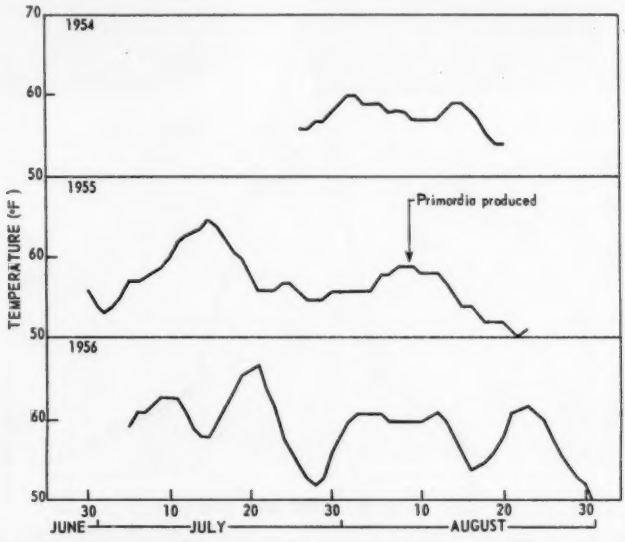

FIG. 7. A five-point moving average of daily mean temperature (6 inches above the ground) in relation to initiation of fruiting in *F. alnicola*.

August, 1955. The failure of the primordia to develop into sporophores is attributed to the dryness of the smooth stump surface. Fully developed sporophores have been observed on stump surfaces only when the surface was rough or had a partial covering of litter (Fig. 8).

The appearance of primordia following a 3-week cool period in 1955 (daily mean temperature below 60° F) suggested that reduced temperature was a responsible factor. This was substantiated later in laboratory experiments. In 1956 the daily mean temperature did not remain below 60° F for more than a week, and no primordia appeared. Primordia were not produced in 1954, although daily mean temperature was at or below 60° F for 3 weeks. The time interval (3 weeks) between exposure of the decayed wood and the period of lower temperature may have been too short for the favorable temperature to be effective. Other observations on fruiting of *F. alnicola* support this. Sporophores have been found on the stump surface of trees that were logged the preceding winter, but not on stumps of trees logged in the same summer.

Initiation of fruiting in nature could not be related to relative humidity.

Fruiting in Culture

F. alnicola

Badcock's (1) method for obtaining fructifications of wood-rotting fungi was tried repeatedly without success, except in one instance. Fruit body primordia frequently formed at the mouth of the test tube or within the medium but did not develop further. One series of Badcock fructification flasks, in which moist air was circulated around the mouth of the test tube, were incubated on a window ledge in December and January. A single fruit body (Figs. 9 and 10) was produced in one of these flasks. No fruit bodies developed in flasks similarly treated in the spring of the same year. As a result of subsequent experiments (see following paragraph), it has now been concluded that the successful production of the single sporophore was a result of the low temperature on the window ledge.

The formation of fruit body primordia in nature after a period when daily mean temperature was below 60° F suggested that a low temperature period was a prerequisite. This was made the basis of the following experiment. Well-established cultures on a soil and wood block medium were incubated at 55° F. A continuous flow of humid air over the culture was provided by passing compressed air through water in a gas dispersion cylinder and through the culture jars. This method was successful and was used repeatedly to produce fruit bodies of *F. alnicola* (Fig. 11). Fruit bodies did not develop in aerated cultures at room temperature, or in unaerated cultures at 55° F. Both reduced temperature and aeration were necessary for fruiting. Occasionally stipes of fruit bodies were formed in cultures kept in the dark, but pilei were rudimentary or lacking. Fruit bodies were formed in various incandescent light intensities from 8 to 50 ft-c. Pilei usually were oriented towards the light source.

The graph of daily mean temperature at the Slave Lake site in 1955 (Fig. 7) shows a peak in mid-July followed by a period of lower temperature, after which fruit body primordia appeared. Fruit bodies were formed in cultures

that were incubated in essentially the same sequence of temperature, that is, first at room temperature (70° to 80° F) and later at 55° F. The necessity for a preliminary period of higher temperature was tested. One of two identical year-old cultures on a soil and wood block medium was incubated at 63° F (*a*) and the other at 45° F (*b*) for 2 months, then both were incubated at 55° F, with continuous aeration and illumination. A robust fruit body was formed in (*a*) after 6 weeks, while in (*b*) a much smaller fruit body appeared only after 11 weeks. It appears therefore that a preliminary period of higher temperature favors fruiting, but is not essential.

F. conissans

Flammula conissans failed to fruit under the same conditions that induced fruiting in *F. alnicola* as described in the preceding section. Primordia did not form at room temperature or in aerated cultures incubated at 55° F. They formed only at 55° F in a still atmosphere. Under these conditions the primordia developed to form small but fully developed fruit bodies. Larger fruit bodies (Fig. 12) developed, however, if the cultures were aerated after primordia had formed.

Light, temperature, and humidity requirements for fruiting were similar in both species, but aeration requirements were different. Fruit body primordia of *F. alnicola* formed at room temperature and at 55° F, in both a still and renewed atmosphere. In contrast, primordia of *F. conissans* formed only in a still atmosphere at a reduced temperature. The location of primordia in soil and wood-block cultures of the two species was also different; invariably the primordia of *F. alnicola* developed on the soil, while those of *F. conissans* developed on the wood blocks. Further development of fruit bodies of *F. alnicola* occurred only in a renewed atmosphere, while those of *F. conissans* developed in either still or renewed air.

Germination of Basidiospores

Freshly discharged basidiospores of *F. alnicola* did not germinate on various media, such as malt, soil extract, and wood extract agars; cellophane over alkaline and acid humus; or malt broth in which the fungus had been grown. Polysporous transfers on malt agar resulted in cultures in one or two instances after a long period of storage. Basidiospores suspended in sterile water and stored at 3° C were plated on malt agar at weekly intervals, and incubated at 7° C. A few spores, roughly 0.2%, germinated after 5 months of cold storage. No spores germinated in later platings.

Basidiospores were kept in cold storage at −7° C as recommended by Kneebone (6). After various periods, spores were plated on malt agar and incubated at room temperature. Germination usually occurred a week after plating. A few spores germinated after 5 weeks of cold storage. Spore germination increased to 0.4% after 10 weeks of cold storage. and remained approximately the same for 13 months. Treatment at lower temperature totally inhibited germination. Spores kept in cold storage at −7° C for 12 weeks were transferred to −18° C for 1½ weeks. Fewer spores germinated following this

treatment than after 13 weeks at −7° C. No spores germinated after 6½ weeks at −18° C.

Spores did not germinate in sterile or unsterile soil after cold treatment.

Cold treatment to induce germination of basidiospores was much more effective with *F. conissans* than with *F. alnicola*. Spores of *F. conissans* germinated after 3 weeks at −7° C, and germination increased to 41% after 11 weeks of this treatment. Germination was much less after 7 months at −7° C and nil after 13 months.

Interfertility Tests

The standard method of determining interfertility type, in which all possible combinations of a series of monosporous cultures are paired, has been followed. The presence of clamp connections after fusion of two monosporous mycelia denotes compatibility, and their absence denotes incompatibility. Rare clamp connections in resulting mycelia are considered to be illegitimate pairings. Following Nobles *et al.* (12), the conventional symbols for the alleles governing interfertility, $A_1A_1B_2B_2$ in tetrapolar species, have been used and assigned in an arbitrary manner.

F. alnicola

Basidiospores from a fruit body produced in culture (isolate DAOM25874) were kept at −7° C for 7 weeks and then plated on malt agar. Isolated colonies which developed on the seeded plates were transferred twice to eliminate ungerminated spores. Nine monosporous cultures obtained in this manner were paired in all combinations. The result of the pairings (Fig. 15) shows that the monosporous mycelia were of four types, and that spores of *F. alnicola* are tetrapolar.

F. conissans

Basidiospores from a fruit body produced in culture (isolate DAOM22888) were kept at −7° C for 5 weeks and then plated on malt agar. Single germinating spores were isolated under the microscope and transferred to malt agar. Twelve cultures were paired in all possible combinations. Figure 16 shows the result of the pairings. Pairings of A_1B_1 and A_2B_2 were fertile and A_1B_1 did not mate with any other group. Pairings of A_2B_2 and A_1B_2, however, were also fertile. The A_2B_2 cultures were, apparently, a mixture of A_2B_2 and A_2B_1. This mixture would not be fertile in itself, but would be fertile in combination both with A_1B_1 and A_1B_2. Additional single germinating spores were isolated, but monosporous cultures of the A_2B_2 groups were not obtained. Nevertheless, the cultures were of four types and it was demonstrated that *F. conissans* is tetrapolar, a condition also reported by Yen (14).

F. alnicola and *F. conissans*

Monosporous mycelia of the four mating types of *F. alnicola* and *F. conissans* (one of the latter was a mixture) were paired in all possible combinations. No fertile combination occurred in any pairings between *F. alnicola* and *F. conissans* (Fig. 17). It is thus demonstrated that these are separate species.

INTERFERTILITY TABLE
Flammula alnicola (Fr.) Kummer
Single Spore Cultures

		A_1B_1			A_2B_2			A_1B_2		A_2B_1	
		3	7	8	1	10	12	5	6	9	
A_1B_1	3		−	−	+	+	−	−	−	−	
	7	−		−	−	+	−	−	−	−	
	8	−	−		+	+	−	−	−	−	
A_2B_2	1	+	+	+		−	−	−	−	−	
	10	+	+	+	−		−	−	−	−	
A_1B_2	12	⊥	−	−	−	−		−	+	+	
	5	−	−	−	−	−	−		+	+	
A_2B_1	6	−	−	−	−	−	+	+		−	
	9	−	−	−	−	−	+	+	−		

INTERFERTILITY TABLE
Flammula conissans (Fr.) Gillet
Single Spore Cultures

		A_1B_1					A_2B_2		A_1B_2			A_2B_1	
		1	2	4	7	9	10	11	3	5	6	8	12
A_1B_1	1		−	−	−	−	+	+	−	−	−	−	−
	2	−		−	−	−	+	+	−	−	−	−	−
	4	−	−		−	−	+	+	−	−	−	−	−
	7	−	−	−		−	+	+	−	−	−	−	−
	9	−	−	−	−		+	+	−	−	−	−	−
A_2B_2	10	+	+	+	+	+		−	+	+	+	−	−
	11	+	+	+	+	+	−		+	+	+	−	−
A_1B_2	3	−	−	−	−	−	+	+		−	−	+	+
	5	−	−	−	−	−	+	+	−		−	+	+
	6	−	−	−	−	−	+	+	−	−		+	+
A_2B_1	8	−	−	−	−	−	−	−	+	+	+		−
	12	−	−	−	−	−	−	−	+	+	+	−	

INTERFERTILITY TABLE
Flammula alnicola (Fr.) Kummer and Flammula conissans (Fr.) Gillet
Single Spore Cultures

		F. alnicola				F. conissans			
		3	10	5	6	1	10	3	8
F. alnicola	3		+	−	−	−	−	−	−
	10	+		−	−	−	−	−	−
	5	−	−		+	−	−	−	−
	6	−	−	+		−	−	−	−
F. conissans	1	−	−	−	−		+	−	−
	10	−	−	−	−	+		+	−
	3	−	−	−	−	−	+		+
	8	−	−	−	−	−	−	+	

+True Clamps ⊥False Clamps

Description of Fruit Bodies

Although descriptions of both species have already been published (7, 8, 9), more complete and directly comparable descriptions are given here.

Flammula alnicola (Fr.) Kummer, Der Führer in die Pilzkunde, p. 82. 1871.

Agaricus alnicola Fr., Syst. Myc. **1**, 250. 1821.

Dryophila alnicola Quél., Enchir. Fung. p. 71. 1886.

Flammula conissans sensu Ricken, Die Blatterpilze, p. 205. 1915.

Pileus 1.5–7 (11) cm, rounded or conical at first, becoming subumbonate, convex, or planoconvex, sometimes with reflexed margin. Clear brown (Munsell color 10YR 5/6) in the button stage with paler margin (2.5Y 8/4), becoming yellow (2.5Y 8/4 at the center to 2.5Y 8/3 at the margin); darker (brown) with age. Viscid in the button stage, becoming dry, glabrous. Traces of a partial veil adhering to the margin for some time. Context yellowish with a watery green line in the flesh just above the lamellae in fresh specimens. Lamellae adnate to subdecurrent, pale yellow, becoming brown due to deposits of spores. Stipe 3–14 cm long, 5–7 (20) mm in diameter, terete, striate, pale yellow (2.5Y 8/3), darker at the base (10YR 5/6). Annulus inconspicuous, superior, fibrillose, sometimes absent.

Spores rusty brown, smooth, 4.5–5.5 (6) × 7–10 μ, ovate ellipsoid, flattened on one side, apiculate (Fig. 13). Basidia hyaline, four-spored, 6–7.5 × 25–30 μ. Pleurocystidia absent. Cheilocystidia clavate or obclavate, 5–8 μ broad. Gill trama of broad, parallel, hyaline hyphae 7–12 μ diameter; vesicular cells 12–20 μ diameter; and occasional narrow hyphae 5–10 μ diameter with yellow contents. Pileus trama composed of a thin pellicle, 60–125 μ thick, of prostrate interwoven or parallel hyphae 3–6 μ diameter; flesh proper of short, broad, interwoven hyphae 10–20 μ diameter without clamp connections. Stipe without caulocystidia.

Habitat.—Usually caespitose on stumps, on roots of windfalls, or in the duff at the base of stumps of white spruce, rarely on living trees. Other hosts are listed under cultural characters.

Specimens examined.—CANADA: Alberta: Seebe, DAOM40076, 25874, on *Picea glauca* var. *albertiana*. Slave Lake, DASFB1385, 1386, 1387, 1388, 1389, on *P. glauca*. Fruit bodies that were developed in culture from isolate DAOM-25874: DASFB1390, 1391, DAOM60219.

Donk (4) pointed out that Kummer had raised *Flammula* to the rank of genus in 1871, antedating Quélet, who had raised it to the genus in 1872. Kummer is, therefore, the authority for the generic name.

The above description agrees closely with that of Kühner and Romagnesi (8) for *Dryophila alnicola*. They note "Odeur spéciale (un peu de bonbons

---

FIG. 15 (Top). Pairing of nine monosporous mycelia of *F. alnicola* in all possible combinations.

FIG. 16 (Center). Pairing of 12 monosporous mycelia of *F. conissans* in all possible combinations. The A_2B_2 group was apparently a mixture of A_2B_2 and A_2B_1.

FIG. 17 (Bottom). Pairing of four monosporous mycelia (isolate 10 of *F. conissans* was apparently not monosporous) of *F. alnicola* and *F. conissans* in all possible combinations.

anglais, H.R., pas d'Hypholoma)". It is not clear what this odor is, but cultures of *F. alnicola* have a penetrating, musty odor which is also evident in decayed wood, but not in the fruit bodies. Kühner and Romagnesi note that pleurocystidia ("cyst. faciales") are absent and "ne pas prendre pour des chrysocystides les vieux éléments hyméniens collapsés, qui sont ± jaunâtres, mais ne fixent pas le bleu lactique". It is noteworthy that these authors state that the forms on hardwoods and softwoods are similar, since the above description of the fruit body is based on collections from softwoods, while the descriptions of European authors were based on collections from hardwoods. They consider *F. conissans* sensu Ricken to be the same as *Dryophila alnicola*.

The description of *Flammula alnicola* by Konrad and Maublanc (7), and also their figure for the mushroom, matches the above description, except that a greenish tinge of the pileus has been noted only rarely by the author. Lange (9) also noted a greenish color at the edge of the pileus. His reference to cystidia, "Cystidia hair-shaped, clavate, apex about 6–7 μ", presumably refers to cheilocystidia. Apart from this, his description is in agreement. The illustration of *Agaricus* (*Flammula*) *alnicola* by Cooke (3) matches the fruit bodies collected by the author.

Flammula conissans (Fr.) Gill., Champ. Fr. p. 525. 1876.

Agaricus conissans Fr., Epicr. Myc. p. 187. 1838.

Dryophila conissans Quél., Enchir. Fung. p. 71. 1886.

Pileus 0.5–2 cm, rounded becoming convex, furfuraceous, then glabrous; yellow (2.5Y 8/6), paler at the margin (2.5Y 8/4). Lamellae subdecurrent, paler than the cap. Veil fugaceous. Stipe 2.5–6 cm long, 2–3 mm in diameter, terete, furfuraceous at first, becoming finely striate; paler than the cap (2.5Y 8/3).

Spores rusty brown, smooth, 4–4.5 × 5.5–8 μ, oval oblong, slightly flattened on one side (Fig. 14). Basidia hyaline, four-spored, 4–5 × 13–20 μ. Pleurocystidia (chrysocystidia)[4] abundant, clavate to ventricose with obtuse apex (some with papillate apex), 8–14 × 24–34 μ with golden yellow contents, deeply staining in lactophenol – cotton blue, embedded in the hymenium. Cheilocystidia filiform, filiform-clavate, or wavy filiform, 3–5 μ diameter. Gill trama of broad parallel hyaline hyphae 5–12 μ broad. Pileus trama composed of a thin, non-viscid pellicle 75–200 μ thick, of prostrate interwoven hyphae, 3–6 μ diameter; flesh proper of short, interwoven hyphae, 5–20 μ broad, without clamp connections. Stipe without caulocystidia.

Habitat.—Kühner and Romagnesi (8) reported this species on the stumps of willows, especially *Salix cinerea*, and Lange (9) reported it as occurring exclusively on *S. cinerea*.

Specimens examined.—Fruit bodies that were developed in culture from isolate DAOM22888 obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland, where it was deposited by Kühner: DASFB1392, DAOM60217, 60247.

[4]See Snell, W. H. and Dick, E. A. 1957. A glossary of mycology. Harvard University Press, Cambridge, Massachusetts. p. 29.

Fruit bodies that developed in culture are generally smaller than those developed in nature, and consequently the upper limit of pileus diameter in this description is less than that in published descriptions. The above description agrees quite well with that of Kühner and Romagnesi (8) except for spore shape, which they state is subcylindric to reniform. Reniform spores were not found. Lange's (9) description agrees except that his measurements of cystidia (cheilocystida) are larger. Lange does not mention the chrysocystidia, which are a striking feature of the hymenium.

The fruit bodies of the two species differ in that *F. alnicola* is larger and darker colored, has a stipe with base darker than the upper part, has a hymenium lacking chrysocystidia, and has larger basidiospores.

Discussion

Different isolates of the same species of wood-rotting basidiomycetes often show variation in growth or other characters. Whether such isolates belong to the same species or related ones is difficult to determine on the basis of cultural characters alone. The relationship of the isolates may be resolved by comparison of fruit bodies produced in culture, and by pairings of monosporous mycelia in heterothallic species with nodose-septate hyphae. Cultures of *F. alnicola* (originally thought to be *F. conissans*) and *F. conissans* are similar except in odor and rate of growth. Major differences in sporophore primordia formation, environmental conditions for fruiting, morphology of the sporophore, and size, shape, and germination of basidiospores are reflected in seemingly minor differences in cultural characters.

Fruit bodies of *F. alnicola* have been observed that could only have been formed from mycelium that has grown up through the soil from infected roots. Conceivably, suitable temperature and humidity conditions could exist below ground, but apparently sporophores are not produced there. Pileus formation as a response to light probably functions as a means of preventing sporophore development below ground.

Since all observed infections have originated in roots below ground, the function of basidiospores is obscure. The conditions under which basidiospores germinated in the laboratory, however, suggests a possible means by which spores may cause infection, and a possible explanation for the occurrence of the fungus only as a root rot pathogen. Basidiospores germinated after cold treatment at −7° C, but not after storage at room temperature or at −18° C. When stored at −7° C, basidiospores remained viable for 13 months. Cold-treated spores did not germinate in sterilized or unsterilized soil. It appears, therefore, that to cause infection, spores must be subjected to a prolonged period of moderately cold temperature followed by a higher temperature for germinating. This condition would restrict infection to the spring season. Basidiospores that lodged on potential infection courts on trunks, such as branch stubs or wounds, could not survive the low winter temperature of the Slave Lake region. In the milder conditions within the soil under cover of

snow, basidiospores could remain viable until spring. The inhibitory effect of soil may function as a device to prevent germination of basidiospores until they reach infection courts on the roots.

Acknowledgments

The author is indebted to Dr. M. K. Nobles for permission to publish the cultural description of *F. alnicola*. The help of Dr. J. W. Groves, who identified the sporophores, supplied the pertinent references, and reviewed the sporophore descriptions, is gratefully acknowledged.

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MICROFUNGI

VI. PIRICAUDA BUBÁK[1]

S. J. HUGHES

Abstract

The type species of *Piricauda* is illustrated and redescribed from the type collection.

In 1918 Bubák revised some species of *Stigmella* Lév. and segregated three species into two new genera, *Stigmopsis* Bub. and *Piricauda* Bub.

The name *Piricauda* was proposed with the following diagnosis, 'Parasitica. Mycelium superficiale, ramosum, septatum, brunneum. Conidiophori solitarii, simplices, 1–3 septati, ex hyphis laxe glomeratis gregatim orientes. Conidia acrogena, pyriformia, clathrato-septata, atrofusca'. A single species *P. uleana* (Sacc. & Syd.) Bub. (≡*Stigmella uleana* Sacc. & Syd.) was included. Bubák gave a brief redescription and added 'Auf den Stellen, wo die Konidien entstehen sollen, bildet sich ein sehr lockeres gekröseartiges Geflecht. . .'

So far as I am aware the genus *Piricauda* remained monotypic until Johnson described *P. pelagica*[2] in May 1958. Moore (1958)[3] redescribed *Piricauda* and the type species was included as *P. paraguayense* (Speg.) Moore (≡*Stemphylium paraguayense* Speg.). In the same year (1959*a*) he added 30 new names to *Piricauda* by transference from other genera and he described five new species. Another new species was added later (Moore 1959*b*).

I have been able to confirm Moore's (1958) synonymy only in part as indicated in the nomenclator below. Moore (1958) and Bubák (1914) included *Stigmella scitula* Syd. (Ann. Mycol. 7: 343. 1909) as a synonym but I have not seen the type collection. Attention is drawn to the fact that in the original descriptions of *Piricauda paraguayense* and its synonyms, as well as in Bubák's (1914) and Moore's (1958) redescription, the long attenuated conidium appendage was regarded as the conidiophore.

Piricauda paraguayense (Speg.) Moore, Mycologia, 50(1958): 691. 1959 (Jan.).

≡*Stemphylium paraguayense* Speg., An. Soc. Cient. Arg. 22:217. 1886.

=*Sporidesmium durantae* Pat., Bull. Herb. Boissier, 3:74. 1895.

=*Stigmella uleana* Sacc. & Syd. in Sacc., Rend. Congr. Bot. Palermo, p. 12. 1902.

≡*Piricauda uleana* (Sacc. & Syd.) Bub., Ann. Mycol. 12:218. 1914.

The redescription that follows is based on the type collection of *Stigmella uleana*, which is illustrated in Fig. 1 and Figs. 2–7.

[1]Manuscript received July 28, 1960.
Contribution No. 90 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.

[2]I denied (in litt. Nov 5, 1957, to Dr. T. W. Johnson) the suitability of the generic name *Piricauda* for the fungus described as *P. pelagica*.

[3]Mycologia, **50** (5) published Jan. 5, 1959.

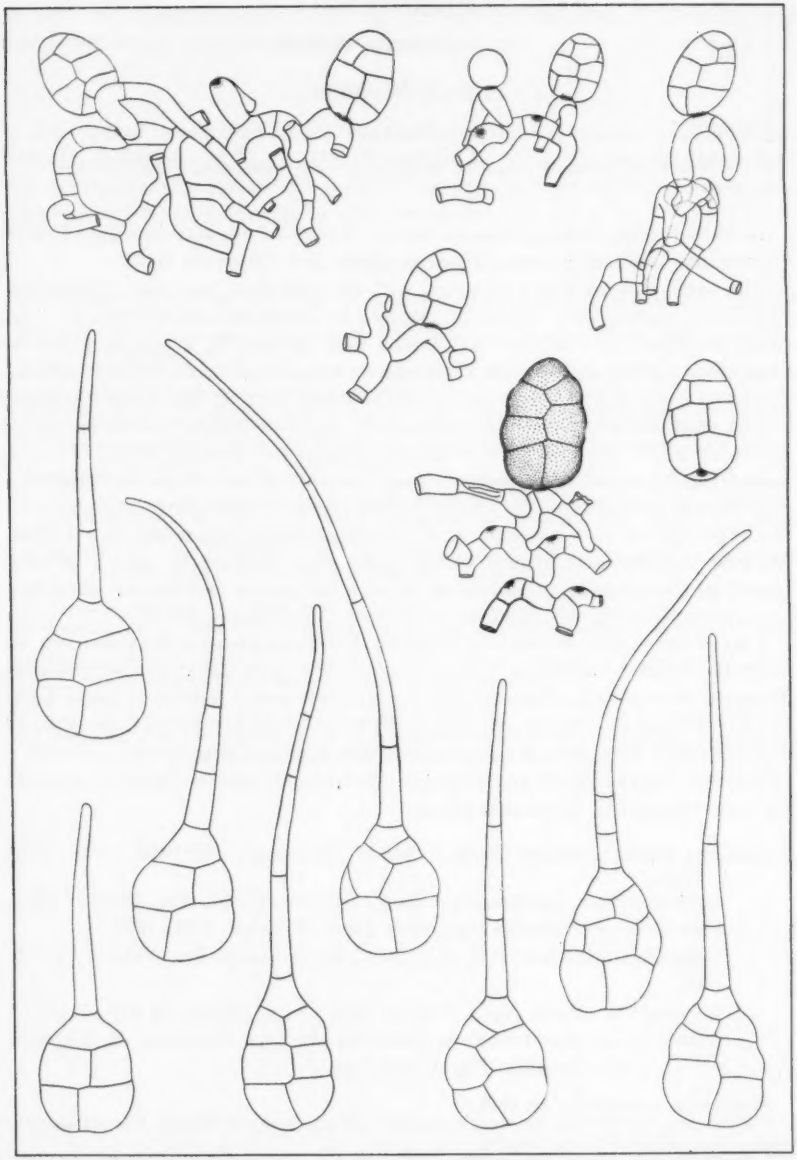

FIG. 1. *Piricauda paraguayense*: conidiophores and conidia from the type collection of *Stigmella uleana*. ×750

The mycelium is apparently entirely superficial and is composed of flexuous, distantly septate, subhyaline to pale brown, smooth, occasionally anastomosing hyphae, 2–3.3 μ wide, which form a network on the leaf surface. Hyphae have been seen overlying the stomatal pores but no penetration has been seen. The growth of hyphae is unaffected by the presence of empty subulate leaf hairs; on the other hand, the hyphae are abundant and closely adpressed around and over the surface of very short-stalked peltate glands and form a dense growth between these and the leaf surface (Fig. 2).

Conidiophores develop in small groups over the colony, occasionally associated with the leaf glands but generally irregularly scattered. From thickened, more closely septate superficial hyphae, arched branches in the form of irregular semicircular loops develop, with their ends reaching and sometimes continuing growth on the leaf surface or occasionally anastomosing with other hyphae. From each of these arched hyphae, and from successive ones, one or two similarly curved branches develop in various planes. The result is a tangle of curved hyphae about 90 μ in diameter but sometimes wider by confluence. Each loop is closely septate at maturity, pale brown to brown, up to 6 μ wide but narrowing somewhat towards the distal end. Each arched hypha is a conidiophore and bears either a conidium initial or a single conidium scar in the form of a pore surrounded by a ring of darkened cell wall.

Conidia develop singly from a pore on the arched conidiophores. Initials are at first spherical and hyaline, then somewhat ovoid and subhyaline to pale brown. They are minutely roughened and soon become septate. Two or three transverse septa are laid down; the terminal cell remains undivided but the lower two or three cells develop usually two longitudinal septa. Towards maturity the apical cell grows out into a straight or gently curved 1–3-septate appendage which is up to 115 μ long, subulate, 4–4.7 μ wide at the base, tapering to 1.3–2 μ wide at the apex; at the immediate base the appendage is pale brown to brown but subhyaline to hyaline for most of its length. The body of the conidium at maturity is thick-walled, dark brown to almost black, more or less smooth-walled, slightly constricted at the septa, and measures 24–34 × 19–24 μ. Conidia fall readily from the conidiophores.

Collections Examined

(1) The type collection of *Stigmella uleana* Sacc. & Syd., in Herb. PAD.[4] '948. In. fol. *Citharexyli* sp. [Verbenaceae]. Brasilia, leg. E. Ule. *Macrosporium* ? an novum genus ? [scr. presumably Ule] *Stigmella uleana* Sacc. sp. n. [scr. Sacc.].' This collection is described above and illustrated in Figs. 1–7. In the original diagnosis the conidiophores were described thus: 'hypha conidiophora simplici, erecta, cylindrica, 1–2-septata, 80–90 × 4–5, e hyalino fuscella, 1–2-septulata.' Bubák drew attention to 'sehr lockeres gekröseartiges Geflecht', which really refers to the true conidiophores.

(2) The type collection of *Stemphylium paraguayense* Speg., in Herb. LPS, No. 13001, '*Stemphylium paraguayense* Speg. Tipo. Paraguay, Peribebuy.

[4]Reference to particular herbaria is made by use of abbreviations found in Index Herbariorum (Regnum Vegatabile, Vol. 6. 1956).

VII. 1883. Leg. Balansa, nro. 3837', on unidentified leaves. The conidiophores are looped as in the previous collection. Conidia measure 19.5–27.5 × 14.5–22.5 μ with the appendage up to 120 μ long.

(3) The type collection of *Sporidesmium durantae* Pat., in Herb. FH, '*Sporidesmium durantae* Pat. [scr. Pat.] Champignons sur les feuilles d'un *Duranta* [Verbenaceae], Cratère de Pululahua, fevr. '92. Lagerh. [scr. presumably Lagerheim]'. The first-formed conidiophores and their branches form the characteristic loops by their growth towards the leaf surface. Conidium production, however, may terminate the growth of subsequent successive branches so that a zigzag structure is produced (Fig. 8). Occasionally, nevertheless, one of these straight branches may proliferate and curve towards the leaf surface, indicating the essential identity of the structure of the conidiophores in this collection and in the two preceding ones. The conidia have two to three but mostly two transverse septa and are pale brown to brown: no black conidia have been seen. They measure 22–33 × 19–26 μ and the appendages are 3.2–5.8 μ wide at the base and up to 110 μ long. A few conidia have been seen with two appendages and frequently the appendage of a detached conidium has been seen to have germinated at the apex and continued growth on the leaf surface. A short, straight, and simple conidiophore bearing a single conidium initial has been seen borne laterally on the appendage of a detached conidium. Despite a few differences this collection is assigned to the name *Piricauda paraguayense*.

For the loan of collections in their keeping I am obliged to Professor C. Cappelletti, Dr. I. Mackenzie Lamb, and Dr. Juan C. Lindquist. Dr. R. A. Shoemaker kindly provided the photomicrographs.

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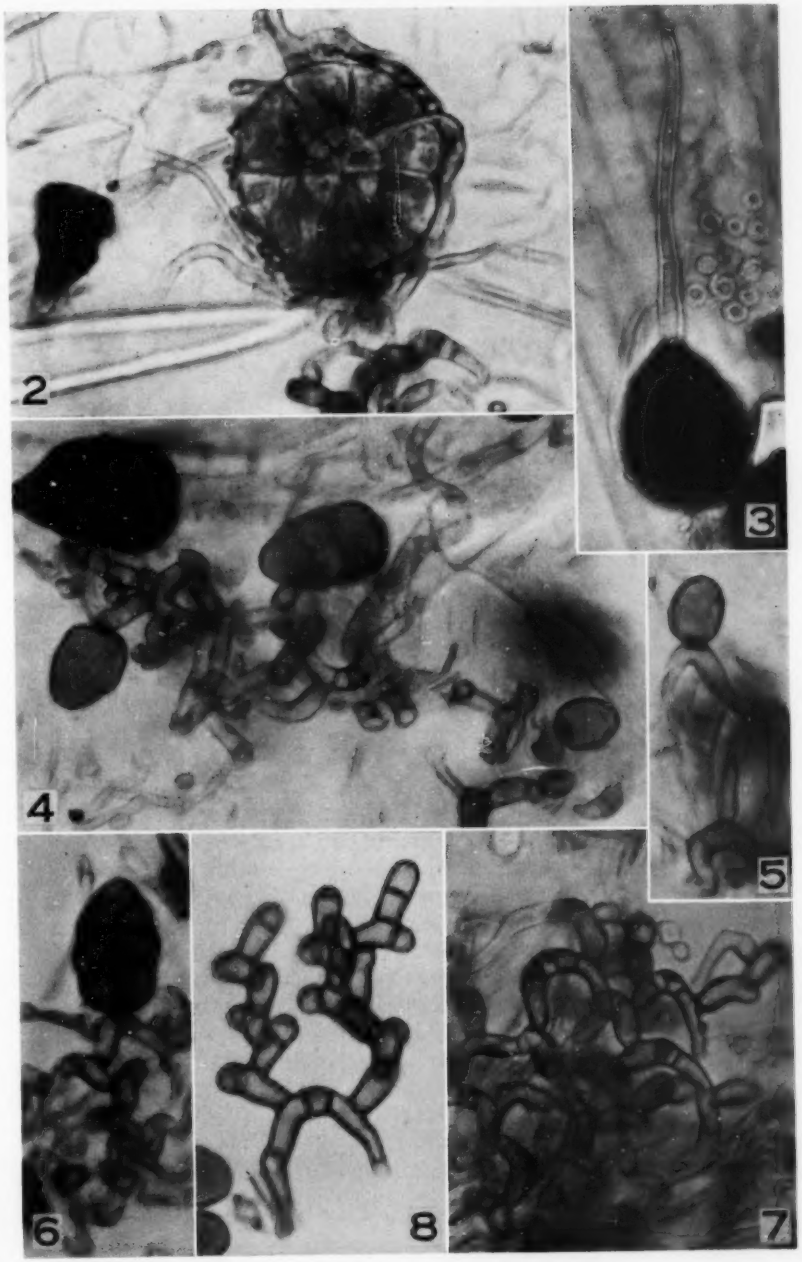
FIGS. 2–8. *Piricauda paraguayense* from the type collections of *Stigmella uleana* (2–7) and *Sporidesmium durantae* (8). Fig. 2, leaf surface with superficial hyphae, hyphae appressed to stalked peltate leaf-gland, and tip of subulate leaf hair. Fig. 3, conidium showing septate appendage with rounded end. Fig. 4, conidiophore loops and young conidia. Fig. 5, young attached conidium. Fig. 6, conidiophore loops with conidium scars and attached conidium (illustrated also in Fig. 1). Fig. 7, tangle of looped conidiophores with conidium scars. Fig. 8, conidiophore loop with zigzags. ×750

PLATE I



Hughes—Can. J. Botany

THE INDUCTION OF APOGAMY IN THE BRACKEN FERN[1]

DEAN P. WHITTIER[2] AND TAYLOR A. STEEVES[3]

Abstract

A new method of inducing apogamy in normal fern gametophytes of *Pteridium aquilinum* has been demonstrated. Entire plants, apparently isolated sporophytic members, and structures of an intermediate nature between sporophyte and gametophyte were produced. The method involved growing gametophytes in sterile culture on a nutrient medium containing a suitable concentration of glucose. A series of experiments was carried out in which the concentration of glucose was varied from 0–8% and the optimum concentration was established to be 2.5%. Prothalli grown in the absence of glucose, which have only their photosynthate as an energy source, produced no apogamous structures. It was found that sucrose, maltose, or fructose could be substituted for the glucose in the induction of apogamy. Four other strains of *Pteridium*, and strains of *Osmunda cinnamomea* and *Adiantum pedatum*, also responded to the sugar treatment by producing apogamous plants. The osmotic pressure of the medium has been shown to have no effect on the induction of apogamy but it does appear to cause the decline in the response at the higher concentrations of sugar. The role of the sugar would therefore seem to be as a respiratory substrate, which in some way favors the induction of apogamy.

Introduction

Since the discovery of apogamy in ferns by Farlow (4) in 1874, there have been many investigations on various aspects of this phenomenon. These investigations have demonstrated two types of apogamy, i.e. the production of a sporophyte, the vascular plant of the asexual generation, from the gametophyte, the non-vascular plant of the sexual generation, without syngamy. The first type, in which the formation of apogamous sporophytes is the rule and one or both of the sex organs may be absent, is called obligate apogamy. Induced or facultative apogamy is the development of apogamous sporophytes from gametophytes which have functional sex organs and normally give rise to sporophytes by syngamy under other circumstances.

The phenomenon of apogamy is an intriguing one for the botanist for two reasons. Since it represents a deviation from the normal life cycle of vascular plants it would seem to provide a favorable place for investigations on the causal factors underlying alternation of generations. From the morphogenetic point of view, the direct origin of a vascular plant from a non-vascular, thalloid fern gametophyte offers exciting possibilities for the study of the orderly development of the plant body. From both points of view, induced apogamy is of greater interest because it does not appear to involve a specific genetic change and its occurrence can, to a degree at least, be brought under experimental control. It is not surprising, therefore, that a number of studies have been carried out on the control of induced apogamy in ferns.

[1]Manuscript received July 13, 1960.
Contribution from the Biological Laboratories, Harvard University, Cambridge, Massachusetts, and the Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan.
[2]Biological Laboratories, Harvard University.
[3]Department of Biology, University of Saskatchewan.

A variety of factors favoring the induction of apogamy in several species of ferns have been reported by previous workers. Heim (6) inferred that upright prothallial growth, that is growth away from the soil surface, which he assumed was related to light factors, was an important factor in the induction of apogamy in *Doodia caudata*. Brown (2) has described reduced vitality of prothalli of *Phegopteris polypodioides* resulting from poor nutrition as leading to the induction of apogamy. Heilbronn (5) has reported that in a race of *Cystopteris fragilis* apogamous sporophytes were produced during the summer while normal embryos were formed during the winter. Higher light intensity during the summer was regarded as the causal factor. The hitherto most successful method of inducing apogamy was that used by Lang (7) in which prothalli were grown for prolonged periods supplied only with capillary water, i.e. watered from below through the soil. By this technique fertilization was prevented and apogamy was induced in several species and varieties of ferns. Lang suggested that the ability for prothallial cells to become meristematic, the prevention of fertilization on the prothallus, and the exposure of the prothallus to direct illumination were important factors in inducing apogamy. Duncan's investigation (3) on *Doodia caudata* corroborated Lang's work but Black (1), Mottier (8, 9), and Steil (11), using the same approach, failed to induce apogamy in other species. It is evident that the causal factors involved in inducing the direct formation of sporophytes from prothalli are not well understood and that further work is necessary. The present paper deals with a case in which the induction of apogamy has been brought under control in sterile culture.

Materials and Methods

Most of the experiments described in this report were carried out with cultured prothalli of the bracken, *Pteridium aquilinum* (L.) Kuhn var. *latiusculum* (Desv.) Underw. (12), which were grown from spores collected in the vicinity of Boston, Mass. In a few cases other ferns were used and they will be described as they are mentioned. The general methods for the culture of fern prothalli on sterile nutrient medium, and particularly those of the bracken, have been described in detail previously by Steeves *et al.* (10). The nutrient medium employed consisted of Knudson's solution of mineral salts supplemented by the addition of minor elements[4] and glucose at varying concentrations, and solidified with 0.5% agar. In certain experimental treatments other carbohydrates were substituted for the glucose. Prothallial colonies were grown

[4]The composition of the minor elements solution is as follows:

H_2SO_4, sp. gr. 1.83	0.5 cc
$MnCl_2.4H_2O$	2500 mg
H_3BO_3	2000 mg
$ZnSO_4.7H_2O$	50 mg
$CoCl_2.6H_2O$	30 mg
$CuCl_2.2H_2O$	15 mg
$Na_2MoO_4.2H_2O$	25 mg
DD H_2O	1000 cc

This solution was devised by Dr. J. P. Nitsch in the laboratory of Prof. R. H. Wetmore at Harvard University. It was added to the medium at a concentration of 1 cc per liter.

on 25 cc of medium in round straight-sided bottles with a diameter of 5.4 cm and a height of 8 cm which were sealed with polyethylene film secured by rubber bands. The cultures were maintained at a temperature of $24 \pm 1°$ C with 12 hours of illumination per day.

Results and Discussion

We have in sterile culture a strain of gametophytes of bracken which are normal in that they will produce sporophytes by fertilization if the cultures are flooded with water. These prothalli have been shown by chromosome counts (n equals 52) to be haploid while the sporophytes resulting from flooding are clearly diploid. However, if these prothalli are placed on a nutrient medium containing a sufficient concentration of sugar, they give rise to a variety of sporophytic structures which are apogamous. These apogamous outgrowths have been shown by chromosome counts to be haploid.

Some of the apogamous outgrowths develop into entire plants which are capable of continued growth (Figs. 1–2). In other cases single leaves arise from the prothallus, apparently in the absence of a shoot apex. Other structures which are intermediate between sporophyte and gametophyte have also been observed. These are elongate, upright, or prostrate cylindrical processes which are either unbranched or dichotomously branched. They contain a vascular strand and usually bear rhizoids and archegonia. The exact details of the origin of the various outgrowths from the prothallus are presently under investigation.

In the main series of experiments, prothalli of the strain mentioned above were cultured on nutrient media containing a range of glucose concentrations. The first recognizable apogamous outgrowths appeared approximately one month after the beginning of the experiment; but the cultures were allowed to grow for 15 weeks before termination since preliminary runs had indicated that the maximum response occurred in this time. In counting the number of apogamous outgrowths in each culture, only entire plants or isolated leaves were included and the intermediate structures were not recorded. The results of this series of experiments are presented in Fig. 3.

No apogamous response of any type has been found on prothalli which were grown in the absence of glucose and have only their own photosynthate as an energy source. At the concentrations below 1% there is a slight response with the production of one apogamous plant in every two or three cultures. A striking increase in apogamy occurs at a concentration of glucose of 1%, with an average of five apogamous plants per culture. The number of apogamous sporophytes per culture steadily increases with the concentration of glucose to a peak of over 12 at 2.5%. Prothalli grown on concentrations of glucose above 2.5% show a decrease in the number of induced plants per culture until at 8% the level is comparable to that of the concentrations below 1%.

Preliminary experiments have shown that sucrose, fructose, or maltose can be substituted for glucose in inducing the formation of apogamous outgrowths. Quantitative differences in response have been noted, however. At a concentration of 2.5% sucrose and fructose gave a slightly smaller response (respectively

10 and 9 plants per culture) than did glucose at the same concentration. Maltose at a concentration of 2% gave a greatly reduced response of one plant per culture in comparison with the same concentration of glucose. On the other hand, it must be remembered that the optimum concentrations for the sugars other than glucose have not been determined so that the reduced response might simply indicate a different optimum.

At the present time apogamous developments have been induced in four other strains of *Pteridium* gametophytes as well as in strains of *Osmunda cinnamomea* and *Adiantum pedatum*. In the *Pteridium* strains and *Adiantum* a higher concentration of glucose was required (3–6%) and duration of exposure was longer. Prothalli were maintained for 4.5 to 11 months on a high concentration of glucose and were transferred to fresh medium 1 to 3 times during this period. In *Osmunda* a single exposure to a concentration of glucose of 5% for 15 weeks was sufficient to induce apogamy. In all cases the response obtained was considerably less than that given by the original strain of *Pteridium* at the optimum concentration of glucose. These experiments, although preliminary, do point out that the induction of apogamy by a high concentration of glucose is not a special case in a single strain of one species. Further experimentation of the same nature may well show that the induction of apogamy by a high sugar concentration is a relatively widespread phenomenon.

It is a matter of considerable importance to know in what way a high concentration of sugar in the nutrient medium causes a thalloid gametophyte to give rise directly to a vascular sporophyte, or some part of such a sporophyte. One possible explanation might be that the induced apogamy is in some way a response to the increased osmotic pressure presented by the higher concentration of glucose in the medium. In order to test this possibility, a series of experiments was carried out with the original strain of *Pteridium* using a medium containing 0.25% glucose supplemented by concentrations of mannitol or Carbowax[5] sufficient to make the final osmotic pressures equal to those of media containing 1 to 2.5% of glucose. Since no apogamous outgrowths were formed, it was concluded that induced apogamy is not basically a response to high osmotic pressure in the surrounding medium. On the other hand, if the optimum concentration of glucose (2.5%) was incorporated in the medium and supplemented by concentrations of mannitol or Carbowax sufficient to make the final osmotic pressure of the media equal to those of media containing 3 to 8% glucose, there was a decrease in the occurrence of apogamy comparable to that observed with higher concentrations of glucose (Fig. 3). It may therefore be concluded that osmotic pressure does become a factor in the decline of the apogamous response to glucose concentrations above the optimum.

An alternative hypothesis to explain the role of sugar in the induction of apogamy is that it functions as a respiratory substrate, thereby increasing the energy available to the organism. This interpretation was inferred by Duncan (3) in considering the influence of direct illumination as a factor in the induc-

[5]A series of water-soluble polyethylene glycols manufactured by the Carbide and Carbon Chemicals Company.

FIGS. 1–2. Apogamous sporophytes of *Pteridium* developing from prothalli in sterile nutrient culture. FIG. 1 ×12. FIG. 2 ×10.

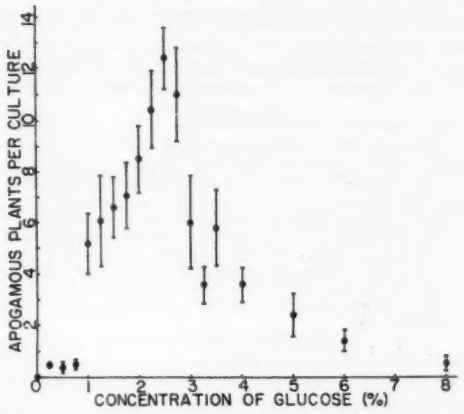


FIG. 3. The influence of glucose concentration in the nutrient medium upon the production of apogamous sporophytes by cultured prothalli of one strain of bracken. Counts were made after 15 weeks. Each point is based upon a minimum of 10 cultures.

tion of apogamy. The fern prothallus is a small, simple, and normally evanescent structure in contrast to the large, histologically complex, and long-lived sporophyte. It does not seem unreasonable to expect that the normal two-dimensional growth of the prothallus, which contrasts sharply with the three-dimensional pattern of the sporophyte, may be related to the limited energy supply normally available to the gametophyte. In accord with this view is the observation that an increased sugar concentration in the nutrient medium almost invariably leads to morphogenetic changes in the gametophyte even in cases in which apogamy does not actually occur. The basic form of the gametophyte on a medium with a low concentration of sugar is the thin, cordate prothallus. On high concentrations of sugar, the prothallus thickens, becoming pad-like, sometimes with a thin margin of normal prothallial tissue. However, it would be an obvious oversimplification of the phenomenon of alternation of generations to regard the major differences between sporophyte and gametophyte as entirely the result of variations in supplies of energy-containing substrates. In the normal life cycle, a variety of controlling mechanisms must be involved in the regular alternation of two contrasting but genetically determined growth patterns. None the less drastic alterations of environment such as are described in this paper may be revealing as to the nature of some of these mechanisms.

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DESCRIPTIONS OF NEW SPECIES OF RHIZOCTONIA[1]

H. K. SAKSENA AND O. VAARTAJA[2]

Abstract

Four new species and one variety are added to the form-genus *Rhizoctonia* from the study of over 60 isolates obtained from forest nurseries in Saskatchewan and Manitoba; these are *Rhizoctonia dichotoma*, *R. endophytica*, *R. endophytica* var. *filicata*, *R. globularis*, and *R. hiemalis*. They are chiefly characterized by chlamydospores in chains produced singly and in sporodochia. The chlamydospore characters were consistent within the species, and are therefore considered of dependable diagnostic value. The sporodochia, however, exhibited considerable variability in character and size depending largely on the substrate, and are of little use for taxonomic purposes.

Introduction

During a study of damping-off in forest nurseries about 700 *Rhizoctonia* isolates have been obtained. Over 60 were selected for a detailed study. Most of the rest were discarded, as they were identical with or close to *R. solani* Kühn or some of the selected isolates. In this study several previously undescribed species and one variety were encountered.

The form-genus *Rhizoctonia* DC. presents a difficult problem for specific classification because many of its members are not known to produce fruiting bodies. This has resulted in incomplete or confusing descriptions of many of the species previously named. It is, therefore, with some reluctance that we add to the substantial number of *Rhizoctonia* species; but we are unable to match many of the isolates with any published description. The type cultures have been deposited in the Centraalbureau voor Schimmelcultures, Baarn, Holland, and the American Type Culture Collection, Washington, D.C., U.S.A. Pathogenicity of the new species ranges from high to nil; this will be the subject of a later paper.

The new species are all characterized by the production of spore-like cells in monilioid chains. For want of a proper name, similar spore-like cells in certain other species of *Rhizoctonia* have been variously referred to in literature as pseudoconidia (5), monilia (3, 4), chlamydospores (12, 13), spores (8), and pseudospores (7, 10). Little information is available on the nature, development, and function of these spore-like cells and their importance in specific differentiation. As discussed later, these spore-like cells are formed by the rounding up of the cells of pre-existing elements of the thallus; they are full of protoplasm and food, and are not deciduous. Because of their origin they are thallospores of the chlamydospore type in accordance with the classification proposed by Vuillemin in 1910 (2, 9) for spores or spore-like bodies of Hyphomycetes. It therefore appears appropriate to retain the name

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chlamydospore for these spore-like cells of *Rhizoctonia*. The chlamydospores are sometimes aggregated into distinct groups, commonly termed sporodochia.

Method of Study

Cultural and vegetative characters including sporodochia, chlamydospores, and sclerotia have been utilized in specific differentiation of species of *Rhizoctonia* in this study. In preliminary investigations, no one medium was found suitable for the proper development of all diagnostic characters. Different culture media were therefore employed, and the descriptions of various vegetative structures of the new species are based on cultures grown on media on which they developed best. Two per cent Difco potato-dextrose agar (PDA) favored the development of cultural and mycelial characters and facilitated their specific differentiation. Two per cent Difco potato-dextrose agar supplemented with 0.75% asparagin (PDAA) induced best sclerotial development. On both these media, the monilioid filaments were produced in good numbers, but further differentiation of their cells into chlamydospores was slow and irregular; the chlamydospores of the same chain or group matured at different periods. Two per cent Difco corn-meal agar without dextrose (CMA) was used for the study of chlamydospores because on it these developed in abundance and attained maturity in 20 to 25 days; the transparency of this medium facilitated direct observations. The inoculated plates were kept at room temperature (25–26° C).

Development of chlamydospores and sporodochia was best studied in connection with pathogenicity tests of various species conducted aseptically in test tubes containing half strength CMA and seedlings. Sporodochia commonly developed in 25 to 30 days on the test tube walls in moist spaces left by shrinkage of the agar, and on exposed roots and stem bases of seedlings. The test tubes were kept in water baths at temperatures varying between 15 and 25° C under fluorescent tubes giving a light intensity of 1500 ft-c.

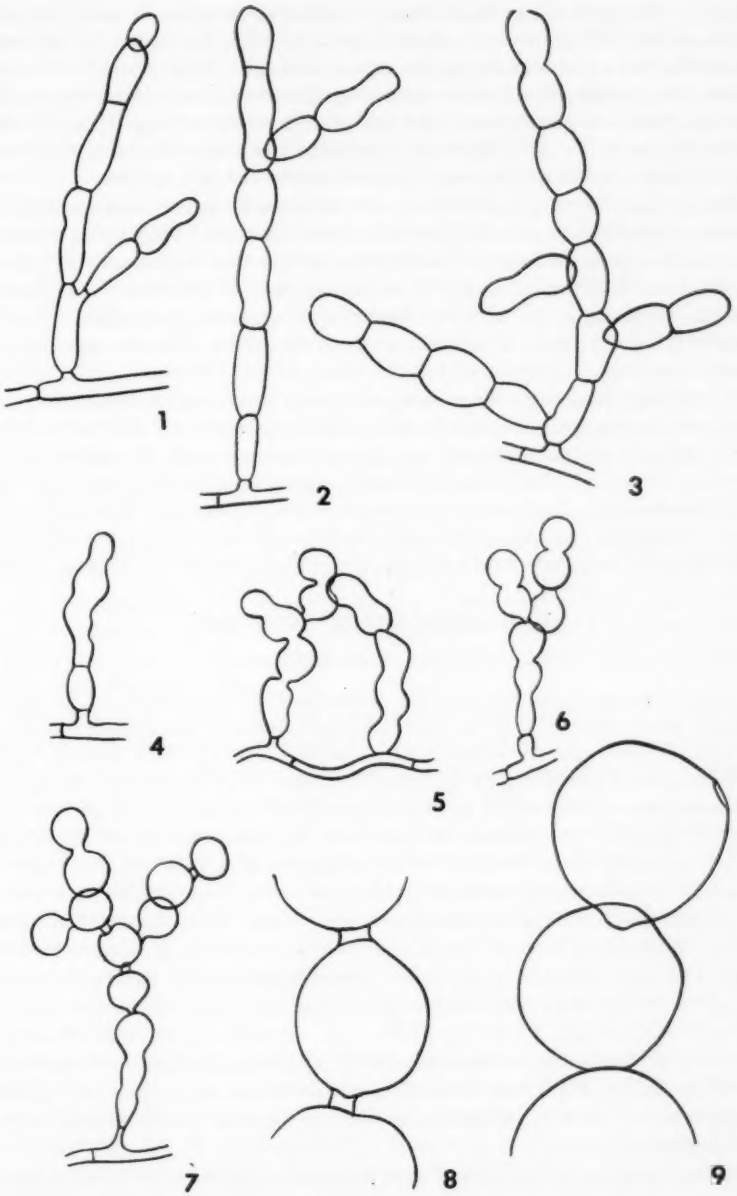
The growth rate of colonies (in terms of average diameter) at different temperatures was studied in 10-cm petri plates containing 25 ml of PDA. These were inoculated with 3.5-mm pieces of inoculum taken from growing margins of 1-week-old colonies. Plates in triplicate for each isolate were incubated at different temperatures.

Color terms that are capitalized are taken from Ridgway (11).

Development of Chlamydospores and their Importance in Specific Differentiation of *Rhizoctonia* Species

The chlamydospores are formed in chains by the inflation or rounding up of the cells of monilioid filaments which arise in the manner of vegetative hyphae (Figs. 1 to 7 and 11). The chains may also grow in length by budding

FIGS. 1 to 9. *Rhizoctonia* species. Figs. 1 to 3. *R. endophytica* isolate 1590. Stages in the development of chlamydospores from the monilioid filaments. Figs. 4 to 8. *R. globularis* isolate 2287. Figs. 4 to 7. Stages in the development of chlamydospores from monilioid filaments. Fig. 8. Chlamydospores separated by slender connections. Fig. 9. *R. hiemalis* isolate 2785. Attachment of chlamydospores in chains. All ×655 except Fig. 8 ×2338 and Fig. 9 ×935.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from the apical cell and by basipetal abscission. Lateral branch chains develop from pre-existing branches of monilioid filaments and from side buds produced by cells of the main chain (Figs. 3 and 7). The chains are variously constricted at septa, delimiting the chlamydospores (Figs. 3 and 9). In some forms, the mature chlamydospores are separated from each other by slender, hyaline, pad-like connections, like the disjunctors of *Albugo* (Fig. 8). The chlamydospores are rich in globular inclusions. The branched chains of chlamydospores occur both singly and in sporodochia in the new species.

The chlamydospores normally do not separate from one another, but the chains usually break into smaller lengths when disturbed while being mounted for microscopic examination. Rarely the mature chlamydospores fall apart as single cells. Whether single or in chains, the chlamydospores germinate within 5–8 hours at 25° C when floated on tap water on a slide. There is usually one germ tube, or sometimes two or three, per chlamydospore, which readily develops into branched hyphae (Figs. 21 and 22).

As already described, the development and structure of chlamydospores and sporodochia were studied on four different substrates. The sporodochia varied greatly in character and size, depending largely on the substrate and to some extent on illumination, humidity, and species. They are therefore not considered of great importance for taxonomic purposes. The chlamydospore characters, on the other hand, were consistent within a species irrespective of the substrate, and are considered reliable for specific differentiation.

Rhizoctonia dichotoma sp. nov.

(Figs. 14 and 15)

Colonia (in agar-agar 2% dextroso *Solani tuberosi*) fulva producit mycelium aerium copiosum lanatum qui nonnunquam vitrum implet. Sero occurrunt in mycelio aerio glomera multa, a fulvis pallide brunnea, laxa, globosa, millimetraliaque. Rami primarii generant ramulos multos, breves, recurvatos qui inter sese anastomosant et reticulum figurant sui generis in pariete vitri et in hypocotyle plantularum inoculatarum. Hyphae vegetatae et super et in medio crescunt modo filicis. Hyphae septatae, in primis hyalinae, deinde brunneae, angulo recto ramosae, gaudent septum primum distans a micris paucis ex origine. Hyphae primariae 6–10 μ crass., ramorum 8–13 μ, aeriae 4–7 μ. Sporodochia in vitro immersa, brunnea, compacta, globosa vel irregularia, 1–2 mm, interdum confluentia. Sporodochia insuper nascent interdum in pariete vitri. Chlamydosporae in primis hyalinae, deinde brunneae, obpyriformes vel clavatae, 30–45 × 20–24 μ, in catenulis dichotomis dispositae. Sclerotia (in asparaginoso dextroso *Solani tuberosi* agar-agar) aeria, globosa vel irregularia, ad 1.5 mm diam. Filamentia sclerotiorum gaudent cellulas irregulares vel doliares, modis variis anastomosantia et cum hyphis vegetatinis intermixta.

In vitro ex plantulis morbosis *Piceae pungentis*, Indian Head, Saskatchewan, Canada.

On PDA, the colony is tan with abundant development of thin, woolly, aerial mycelium which sometimes fills the culture tubes and plates. Numerous tan to light brown, loose, round hyphal balls, 1 mm in diameter, appear later within the aerial mycelium. The lateral branches of main aerial hyphae produce numerous, short, recurved, secondary branches on either side, which anastomose at their tips with similar hyphae at point of contact to form a characteristic net-like pattern on the test tube wall (Fig. 14). Similar hyphal nets were formed on the hypocotyls of seedlings inoculated with this isolate.

The vegetative hyphae, on and in media, show fern-like growth. The hyphae are septate, hyaline when young, later brown, branching at right-angles, with the first septum placed a few microns beyond the point of origin. The main hyphae are 6–10 μ wide, side branch hyphae 8–13 μ, and aerial hyphae 4–7 μ.

The sporodochia in culture (PDA, PDAA, CWA) are submerged, white, later brown, compact, round to irregular, 1–2 mm in diameter, at times confluent. In 6-week-old cultures on CMA, the sporodochia may become hard and wax-like resembling sclerotia; however, they retain their original structure with no intertwining of the chlamydospore chains with the vegetative mycelium. In test tubes with seedlings, sporodochia produced against the wall are brown, round, 1–2 mm in diameter.

The chlamydospores (Fig. 15), produced in dichotomously branched chains, are hyaline when young, later brown, obpyriform or clavate (widest at the summit), 30–45 $\times$ 20–24 μ in diameter.

Sclerotia, observed only on PDAA after 6 weeks of growth, are aerial, round to irregular, and up to 1.5 mm in diameter. They consist of filaments of short, irregular to barrel-shape cells, variously anastomosed and intertwined with vegetative mycelium.

The colony is fast-growing with temperature optimum at 25° C (Table I).

Isolated in culture from diseased *Picea pungens* Engelm. seedlings grown in mixture of sand and old seed-bed soil in greenhouse experiment, October, 1956, Indian Head, Saskatchewan, Canada (No. 2486, Type).

R. dichotoma is close to *R. solani* in cultural appearance, but is readily distinguished: by the presence of sporodochia with chlamydospores in dichotomously branched chains, the chlamydospores being larger and differing in shape from the barrel-shape cells of *R. solani*; by copious development of light tan aerial mycelium with short recurved anastomosing hyphae; by light colony color which does not turn brown as in *R. solani*; by rare production of sclerotia which are aerial and smaller than in *R. solani*; and by faster growth.

This species is perhaps closest to *R. borealis* Curtis, which has been isolated from orchid roots as a mycorrhizal fungus and described briefly without Latin diagnosis (8). Unfortunately a culture of *R. borealis* was not available for comparison, but *R. dichotoma* differs from it in having smaller chlamydospores, short recurved anastomosing hyphae and sclerotia, and in its ability to cause severe damping-off of seedlings of various genera.

R. dichotoma is easily distinguished from *R. stalhii* Burgeff (4) (an orchid endophyte which also has short, crooked, anastomosing hyphae) by its tan colony color, brown hyphae, larger chlamydospores, smaller sclerotia, and pathogenicity.

Rhizoctonia endophytica sp. nov. variety *endophytica*

(Figs. 1 to 3, 16, and 17)

Colonia (in agar-agar 2% dextroso *Solani tuberosi*) hyalina cum mycelio radiali superficiali et mycelio aerio humile lanuginoso albo. Sero occurrunt glomera alba globosa, 1 mm, huc illuc congregata in hyphis aereis. Colonia dorso Light Ochraceous Buff. Hyphae vegetativae septatae, in primis hyalinae, deinde brunneae, angulis quadratis ramosae, in jugo constrictae, gaudent septum primum distans micris paucis a jugo. Hyphae primariae 3–5.5 μ crass. et ramorum brevium lateralium 4–7 μ. Sporodochia in vitro immersa, in primis hyalina, deinde ochroleuca, a globosis et 0.5 mm confluentia in molem plurimillimetralem. Sporodochia insuper nascent interdum in pariete vitri cum sunt ochroleuca, granularia, laxa, globosa, ad 0.75 mm diam. Chlamydosporae hyalinae, (collective brunneae), doliformes, 18–22 × 9–12.5 μ, in catenulis rectis elongatis ramosis 12–20 cellularum dispositis. Sclerotia (in asparaginoso dextroso *Solani tuberosi* agar-agar) aeria vel superficialia, a globosis irregularia, 5–10 mm long., Bone Brown intus, Cinnamon Buff vel Pinkish Buff extus, vestita pallidis lanatisque hyphis.

In vitro ex plantulis *Pini banksianae*, pago Big River dicto, Saskatchewan, Canada.

On PDA, the young colony is hyaline with radial growth of surface mycelium. From this arise irregular, low, cottony, white, aerial hyphae which later develop white, round hyphal balls, 1 mm in diameter in scattered groups. In old cultures, reverse of colony is Light Ochraceous Buff.

The vegetative hyphae are septate, hyaline, becoming brownish with age, branching at right angles, with a constriction at the point of junction, and the first septum placed a few microns beyond this. The hyphal branches are frequently anastomosed. The main branch hyphae are 3–5.5 μ and the short side branch hyphae 4–7 μ wide.

The sporodochia in culture (PDA, PDAA, CMA) are submerged, hyaline when young, later cream colored; they vary from round, 0.5-mm spots to confluent irregular masses, several millimeters in length. In occasional cultures, chlamydospore chains may also occur on the surface of media in minute, white plaques. The aerial chains stand out freely in the air in all directions. Sporodochia development was best seen against the walls of test tubes with seedlings; there they develop as cream-yellow, granular, open-textured, round bodies, up to 0.75 mm in diameter, in scattered groups. They are chiefly formed along main hyphal branches, giving a crow's-foot pattern. Straight, long, branched chains of 12 to 20 chlamydospores usually radiate from the center of each sporodochium (Fig. 16). In the presence of continued

condensed moisture in the tube, the sporodochia acquire a smooth surface of intertwining vegetative hyphae.

The chlamydospores (Figs. 3 and 17) are hyaline, brownish in mass, doliform, 18–22 × 9–12.5 μ in diameter.

Sclerotia developed only on PDAA are aerial or surface borne, round to irregular, 5–10 mm in diameter. They are Bone Brown inside, Cinnamon Buff to Pinkish Buff outside, and have a woolly mantle of aerial hyphae. These large sclerotia developed, one or two per flask, by the coalescence of a number of smaller sclerotia. In certain isolates of this species (Nos. 2203, 2320, and 2710) several 2-mm sclerotia occurred in groups in masses of aerial hyphae, while in others (Nos. 2203, 2710, 1293) they occurred also against the glass wall.

The colony is fast growing with temperature optimum at 25° C (Table I).

Isolated in culture from nursery-grown coniferous seedlings of the following species: (*a*) *Pinus banksiana* Lamb. from dieback patches in 1-year-old seedbeds, May 26, 1954, Big River, Sask., Canada (No. 1590, Type); from damping-off, Big River, Sask., Canada, 1954 (No. 1673) and 1956 (No. 2320). (*b*) *Pinus sylvestris* L. from 3-year-old overdense seedbeds, Indian Head, Sask., Canada, 1953 (Nos. 1293 and 1305); from damping-off and young seedlings in greenhouse experiments with virgin soil (No. 2746) and old nursery soils (No. 2740). (*c*) *Picea glauca* (Moench) Voss from damping-off, Big River, Sask., Canada, 1958 (No. 2860) and Indian Head, Sask., Canada, 1953 (No. 1302). (*d*) *Picea pungens* from damping-off, Indian Head, Sask., Canada, 1955 (No. 2203).

Two of these isolates were from seedlings considered healthy (Nos. 1293 and 2740). Inoculation experiments suggested that this species is an endophyte in roots of various conifers and angiosperms. It forms packets of chlamydospores filling the cortical root cells.

The wider hyphae, long straight chlamydospore chains, and dark large sclerotia distinguish *R. endophytica* from the orchid endophyte, *R. goodyerarepentis* Costantin (6), which it resembles closely. *R. endophytica* is close to *R. choussii* Crandall & Arillaga (7) in chlamydospore size, but differs in having aerial mycelium, sporodochia, sclerotia, and thin hyphae.

TABLE I

Diameter in mm of colonies of *Rhizoctonia* species after 6 days of growth on PDA at various temperatures

Species and isolate No.	Temperature, °C					
	7	12	17	22	25–26	30
R. dichotoma (2486)	+	11	65	94	Over 95	71
R. endophytica (1590)	+	20	66	88	Over 95	92
R. endophytica var. *filicata* (1080)	0	9	33	57	76	71
R. globularis (2287, Type)	+	11	32	45	47	42
R. globularis (2324)	0	8	31	40	42	+
R. hiemalis (2785)	22	32	70	77	84	12

NOTE: 0 = No growth; + growth not measurable.

R. endophytica is easily distinguished from related orchid symbionts that produce chlamydospores, namely, *R. arachnion* Burgeff, *R. aerea* Burgeff, *R. floccosa* Burgeff (3), and *R. moniliodes* Curtis (8), by its larger chlamydospores and the presence of sporodochia and sclerotia; from *R. gracilis* Burgeff, *R. stahlii*, *R. sclerotica* Burgeff, and *R. asclerotica* Burgeff (4) by its smaller chlamydospores; and from *R. repens* Bernard (1, 4) by its chlamydospore characters and the presence of sclerotia and aerial mycelium. The rest of the orchid rhizoctonias (1, 3, 4, 8) and other chlamydospore-producing species (5, 10, 12, 13) have chlamydospores of different sizes and (or) colored hyphae.

Rhizoctonia endophytica var. *filicata* var. nov.

(Fig. 18)

Colonia (in agar-agar 2% dextroso *Solani tuberosi*) hyalina cum mycelio immerso filicoideo et mycelio superficiali albo tunui recumbente et huc illuc appresso. Colonia dorso Cinnamon Buff in aetate. Cristalli numerosi rhomboidei apparent in medio. Hyphae et sporodochia sicut typica, sporodochia tamen interdum nascent in pariete vitri. Chlamydosporae in primis hyalinae, deinde brunneae, doliformes, 19.5–25 × 10.5–13.5 μ, in catenulis elongatis ramosisque dispositis. Catenulae saepius ad medias recurvatae, intertactae et anastomosantes. Sclerotia mihi ignoti.

In vitro ex plantulis morbosis *Pini banksianae*, pago Big River dicto, Saskatchewan, Canada.

On PDA, the young colony is hyaline with fern-like growth of mycelium. The surface of the colony is later covered by a thin layer of recumbent, white mycelium, appressed at places, and climbing on the glass walls. In old cultures, reverse of colony is Cinnamon Buff. Numerous rhomboid crystals are present within the medium.

Mycelial and sporodochial characters in culture are as described for the type variety. Sporodochia have not been observed to develop against the wall of test tubes with seedlings.

The chlamydospores (Fig. 18) are hyaline when young, later light brown, doliform, 19.5–25 × 10.5–13.5 μ in diameter. The chains of chlamydospores are long (up to 25 spores), usually recurved from the middle, and intertwined and anastomosed with their counterparts.

Sclerotia have not been observed on any media.

Optimum temperature for growth of colony is 25° C (Table I).

Isolated in culture from damped-off seedlings of *Pinus banksiana*, Big River, Sask., Canada, September, 1954 (No. 1080, Type). This isolate produced root rot on seedlings of various conifers and angiosperms in inoculation experiments.

Variety *filicata* differs from the type variety in having fern-like growth of submerged mycelium, poor development of aerial mycelium, slightly larger chlamydospores, and no sclerotia. The chains of chlamydospores of variety *filicata* are often recurved; they intertwine and anastomose with similar

chains of the sporodochium. While variety *endophytica* is an endophyte of root cells which become filled with packets of chlamydospores, variety *filicata* causes root rot and its mycelium ramifies within the host cells. It does not produce chlamydospores within the host cells, and has a slower growth rate than the type variety.

Rhizoctonia globularis sp. nov.

(Figs. 4 to 8, 19 to 22)

Colonia (in agar-agar 2% dextroso *Solani tuberosi*) incolor aequaliter crescit radiale mycelio sericeo hyalino zonato, zonis intervallis 0.4 mm. In aetate mutatur colonia et mycelium fit appressum translucens et lucidum. Mycelium aureum deest. Hyphae vegetativae septatae, hyalinae, 2.5–5 μ crass., ramis saepe ortis quadratis saepius angula 45°, gaudent septum primum distans micris paucis a jugo. Sporodochia in vitro immersa variant in medio. Si in dextroso *Solani tuberosi* agar-agar, confluent in plagis irregularibus Light Buff ad 2 cm long. Si *Zeae mais*, sporodochia alba, opaca, globosa, millimetralia, interdum confluent. Aliquandum sporodochia alba, granularia et millimetralia nascent et in pariete vitri et ad basas plantularum. Chlamydosporae hyalinae, globosae, 9–11 μ diam., in catenulis brevibus ramosis, moniliformibus, 3–8 cellularum dispositis. In aetate chlamydosporae discedent discis tenuibus hyalinisque. Sclerotia mihi ignota.

In vitro ex raciculis mycorrhiziferis *Pini banksianae*, Big River, Saskatchewan, Canada.

On PDA, the colony is colorless and has uniform surface growth of silky, hyaline, radial mycelium with fine zones 0.4 mm apart. When old, the surface mycelium is appressed, and the colony becomes smooth, translucent, and glistening. Aerial mycelium is absent.

The vegetative hyphae are septate, hyaline, 2.5–5 μ wide, branching often at nearly right angles but more commonly at a 45° angle, with the first septum placed a few microns beyond point of origin.

The sporodochia in culture are submerged and very variable on different media. On PDA and PDAA, they are confluent in irregular Light Buff patches up to 2 cm long; on CMA, they are white, opaque, usually round, 1 mm in diameter, sometimes confluent. Chains of chlamydospores in minute, white plaques are produced occasionally on agar surface, either by the surface mycelium or sporodochia underneath. In test tubes with seedlings, the chlamydospore chains occur distributed unevenly or in minute groups in a continuous white mantle of vegetative mycelium on the tube wall and exposed roots and hypocotyls of seedlings (Fig. 19). In the presence of condensed moisture in test tubes, the sporodochia formed on walls are granular, white, and up to 1 mm in diameter.

Chlamydospores (Figs. 7 and 20), produced in short, branched, monilia-like chains of 3–8 cells, are hyaline, globose, 9–11 μ in diameter. Mature chlamydospores are separated from each other by hyaline, slender, pad-like connections which, unlike the chlamydospores, do not stain with cotton blue (Fig. 8). When an aerial tuft of chlamydospores is disturbed gently in

mounting medium, the chains readily fall apart into smaller lengths and as single cells (Figs. 21 and 22).

Sclerotia have not been observed.

Optimum temperature for growth of colony is 25° C (Table I).

Isolated in culture from mycorrhizal rootlets of seedlings of *Pinus banksiana*, forest nurseries, Big River, Sask., Canada, 1956 (No. 2287, Type) and Pineland, Manitoba, Canada, 1956 (No. 2616), and from damped-off seedlings of *Picea glauca*, Indian Head, Sask., Canada, 1956 (No. 2324).

Isolate 2324 differs from *R. globularis* in certain respects, but is referred to that species because of similarity in mycelial and chlamydospore characters, and in production of sporodochia on PDA, host surface, and walls of test tubes with seedlings (Fig. 19). It differs from the type isolate in the absence of zonate rings, in the presence of fine, silk-like aerial mycelium, and in the production of compact, waxy, cream-colored sporodochia 1 mm in diameter within CMA in groups around the central piece of inoculum and along the margin of plates within 1 cm of the perimeter. The chlamydospore chains are longer (6 to 12 or more cells) than in the type isolate, with chlamydospores usually in various stages of differentiation and development.

The radial growth rate of the colony at temperatures below 30° C is closely paralleled in the type isolate and isolate 2324, with optimum at 25° C (Table I). At 30° C, however, while the type grew well, there was little growth in isolate 2324.

R. globularis is close to only one of the many orchid rhizoctonias, namely *R. repens* as described by Burgeff (4) and Curtis (8). The type culture was compared with a duplicate culture of *R. repens* obtained from the Centraal-Bureau voor Schimmelcultures. The conspicuous radial growth of surface mycelium with fine zones and the smaller chlamydospores with their typical mode of attachment in a chain distinguish *R. globularis* from *R. repens*, as well as from closely related *R. choussii*.

The absence of aerial mycelium and sclerotia and the characteristics of its chlamydospores separate *R. globularis* from other chlamydospore-producing species of *Rhizoctonia*, described from Italy by Castellani (5) and by Sappa and Mosca (12, 13).

Basidia with tiny sterigmata and basidiospores were observed twice in CMA cultures of isolate 2287. However, this did not provide enough material for the identification or description of the perfect stage. Repeated efforts to produce the perfect stage again have so far failed. This difficulty is similar to that experienced commonly with some isolates of *R. solani* (*Pellicularia filamentosa* (Pat.) Rogers).

Rhizoctonia hiemalis sp. nov.

(Figs. 9–13, 23, and 24)

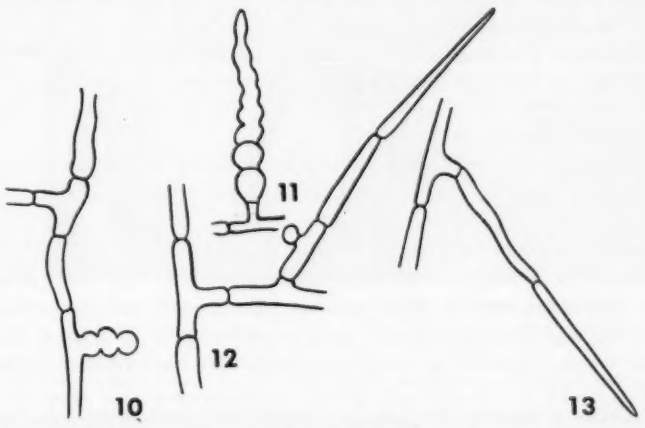
Colonia (in agar-agar 2% dextroso *Solani tuberosi*) atrobrunnea, mycelio aereo mediocriter evoluto, pallide brunneo. Hyphae vegetativae saepe ad septas constrictae, quadrater ramosae, guadent septum primum distans

micris paucis a jugo. Hyphae in primis hyalinae, deinde brunneae continent globos oleagineos crassos atque res granulosas. Rami primarii 5.5–8 μ crass., secundarii dimorphi 7–10 μ vel 3–5 μ crass. Hyphae nonnunquam crassitudine variae et ramis attenuatis. In aetate hyphae praecipuae et hypae aeriae minute verrucosae. Rami saepe anastomosantes, nonnunquam apicibus in cochleam 4–6-ter involutis. Sporodochia in vitro superficialia vel immersa, in primis alba, deinde brunnea, irregularia vel globosa, 0.5–2 mm diam., interdum in moles crassitudinis variae confluentia. Catenulae chlamydosporarum natae in mycelio vel in hyphis rameis brevibus 10.5–16 μ crass., irregulariter gemmantes. Chlamydosporae brunneae, globosae, 24.5–35.0 × 22.0–32.5 μ diam., plenea globis oleagineis crassis, ad apices paullum compressae. Sclerotia, interdum generantur in agar-agar dextroso *Solani tuberosi*, convexa, intus atro-brunnea, extus griseo-brunnea, globosa vel irregularia, 2 mm long vel in moles majores confluentia. Sclerotia texta sunt ex fasciculis compactis catenularum anastomosantibus cum hyphis vegetativis atro-brunneis.

In vitro ex aciculis emortuis caducis *Pini sylvestris* cultae. Lecta parum post nivem dilapsam apud Saskatoon, Saskatchewan, Canada.

On PDA, the colony is dark brown with moderately developed pale-brown aerial mycelium.

The vegetative hyphae are septate, often constricted at septa, branching at nearly right angles, with the first septum placed a few microns beyond point of origin (Figs. 10 to 13). The hyphae are hyaline when young, later brown and full of large oil globules and granular material. The main branch hyphae, 5.5–8 μ wide, produce two types of side branch hyphae, 7–10 μ and 3–5 μ wide. The hyphae at times vary in width (Fig. 10). The side branch hyphae often taper to a fine tip (Figs. 12 and 13). The main hyphae and



FIGS. 10 to 13. *Rhizoctonia hiemalis* isolate 2785. Fig. 10. Hypha of irregular width. Fig. 11. Monilioid filament. Figs. 12 and 13. Normal hyphae with tapering branch hyphae. All ×229.

aerial hyphae in old cultures acquire a finely warted surface (Fig. 23) which appears to be due to a deposit of dried granular matter. Bridge fusion and anastomoses of hyphal branches are common. The ends of the hyphal branches are occasionally spirally coiled 4 to 6 times.

The sporodochia in culture (PDA, PDAA, CMA) are mostly submerged, white when young, later brown, irregular to round, 0.5–2 mm in diameter, and sometimes confluent in masses of variable sizes. Minute, greyish-brown, superficial sporodochia develop frequently in old cultures with chlamydospore chains protruding freely in the air in all directions.

The chlamydospores, in irregularly budded chains, occur directly on the mycelium or on short, 10.5–16 μ thick hyphal branches. The chlamydospores (Figs. 9 and 24) are brown, globose, 24.5–35.0 $\times$ 22–32.5 μ in diameter, full of large oil globules, and frequently anastomosed at point of contact with chlamydospores of the group.

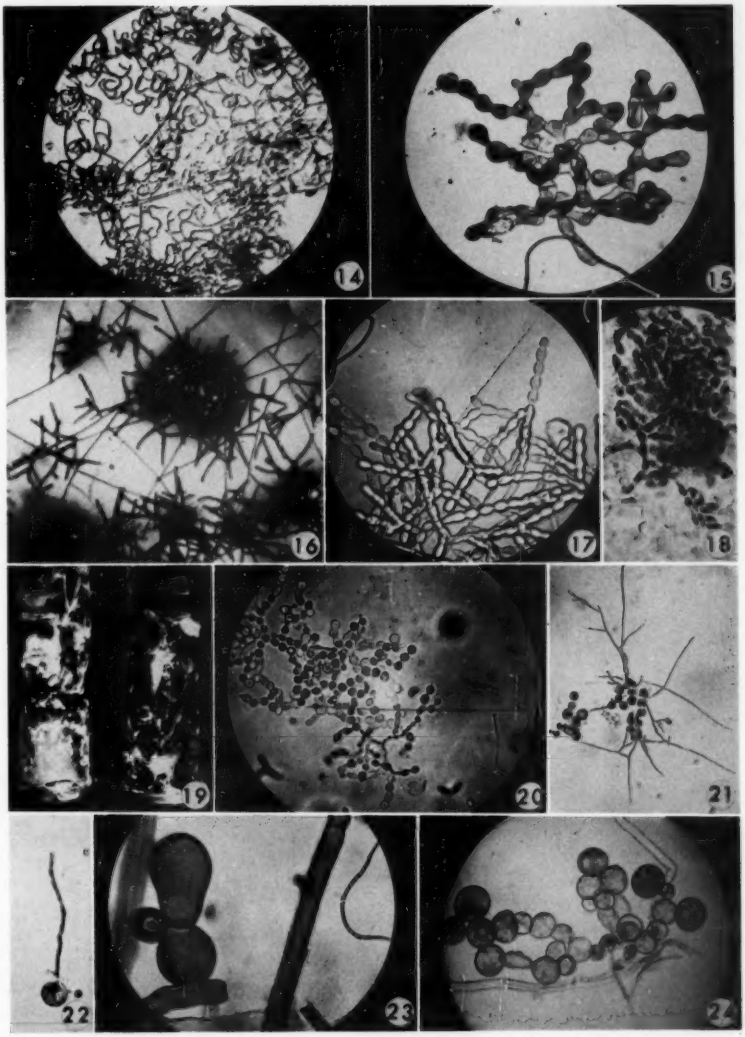
Sclerotia are convex, round to irregular, up to 2 mm long; sometimes a number of them are confluent in larger masses several millimeters in length. They are dark brown inside and greyish brown outside, and consist of compact masses of variously anastomosed chlamydospore chains with intertwining, dark brown, coarse, vegetative hyphae. These sclerotia were observed on 2-month-old PDA culture plates previously used in the study of temperature effect on the colony growth and left at 17° C. On these plates there was abundant production of greyish-brown, superficial sporodochia, aggregated in the outer 2 cm of the plates. Some of these later thickened to a spherical form resembling small sclerotia. True sclerotia were produced mostly along the margins of the plates, pressed against the walls. The development of sclerotia in this species appears to be influenced by temperature since they were not produced at 22° C and over on PDA. Furthermore, sclerotia failed to develop at room temperature (25–26° C), even on PDAA, which stimulated sclerotia development in other species.

The optimum temperature for growth of colony is 25° C (Table I) with a sharp decline at 30° C, at which temperature the growth rate is much slower than at 7° or 12° C. This isolate shows greater tolerance of low temperatures than other isolates studied.

Isolated in culture from mold on pine litter under *Pinus sylvestris* plantation soon after melting of snow, March 21, 1958, Saskatoon, Sask., Canada (No. 2785, Type).

R. hiemalis is readily distinguished from *R. solani* and *R. dichotoma*, which have somewhat similar colony color and appearance, by the presence of globose chlamydospores, the absence of fern-like growth, and by the characteristics of its vegetative mycelium, that is, constriction of hyphae at septa, irregular width, tapering of branch hyphae, and the presence of large oil globules.

R. hiemalis is close to *R. anomala* Burgeff (4) in chlamydospore size, but differs from this species by its brown color and other characteristics of its vegetative mycelium. Burgeff's description of *R. anomala* is brief and without



FIGS. 14 to 24. *Rhizoctonia* species. Figs. 14 and 15. *R. dichotoma* isolate 2486. Fig. 14. Short, recurved, anastomosing aerial hyphae (×51). Fig. 15. Chlamydospores in dichotomously branched chains (×127). Figs. 16 and 17. *R. endophytica* isolate 1590. Fig. 16. Sporodochia on the walls of test tubes as seen under L.P. (×62). Fig. 17. Chlamydospores in long, straight chains (×127). Fig. 18. *R. endophytica* var. *filicata* isolate 1080. Chlamydospores in anastomosing chains (×127). Fig. 19 to 22. *R. globularis*. Fig. 19. White mantle of hyphae and sporodochia on the test tube wall and hypocotyls of seedlings (left, isolate 2287; right, isolate 2324). Fig. 20. Chlamydospores in short, branched chains (×127). Figs. 21 and 22. Germinating chlamydospores. Figs. 23 and 24. *R. hiemalis* isolate 2785. Fig. 23. Old hypha with finely warted appearance (×506). Fig. 24. Globose chlamydospores (×127).

Latin diagnosis. No details are given to suggest that this species would possess the very conspicuous characteristics described above for *R. hiemalis*.

Acknowledgment

Grateful acknowledgment is made to Dr. B. Boivin, Plant Research Institute, Ottawa, for providing the Latin diagnoses.

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ADDITIONAL DIAPORTHALES ON TWIGS FROM ONTARIO[1]

JAMES REID[2] AND R. F. CAIN[3]

Abstract

The following fungi are newly reported from Ontario: *Cryptosporella paucispora* (Pk.) Berl. & Vogl., *Diaporthe taleola* (Fr.) Sacc., *Diaporthe leiphaemia* var. *raveneliana* (De Thüm. & Rehm) Wehm., *Apioporthe anomala* (Pk.) Höhn., and *Valsa salicis* (Fuckel) Winter.

The status of the family Cryptosporellaceae sensu von Arx and Müller is briefly discussed.

Introduction

In studying collections made from Ontario tree species, fungi of special interest are frequently encountered. These organisms may be important because they assist in elucidating family relationships, or because they have never been previously recorded from this Province or from Canada. Five such species, all representatives of the Diaporthales sensu Gaümann (3), are reported on in this paper.

Names of herbaria are abbreviated according to the third edition of the Index Herbariorum.

Cryptosporella paucispora (Pk.) Berl. & Vogl. Sylloge, Additamenta ad Vol. I–IV. 1886

Valsa paucispora Pk. N.Y. State Mus. Rept. 33:33. 1883

Cryptospora paucispora (Pk.) Ell. & Ev. North Am. Pyr.: 529. 1892

Perithecia single or aggregated in clusters of as many as 20, often forming a linear series, subglobose and usually depressed from below, grayish-black, smooth, bare, embedded in the cortex, 300–400 μ in diameter. Neck black, about 300–400 μ in length and 150 μ in diameter, lined with a layer of periphyses lying parallel and extending obliquely upward, surrounded by stroma forming a clypeus, projecting conspicuously beyond the stroma to form a black, smooth papilla with a small circular ostiole. Stroma forming a compact, light yellowish-brown mass composed of yellowish, thick-walled fungous cells, exposed by the rupturing of the outer periderm of the cortex, and merging with neck tissue without a sharp line of demarcation. Clypeus fusing together a cluster of necks. No dorsal or ventral dark lines apparent in the cortex or wood. Peridium of perithecium thin, membranaceous, consisting of two layers. Outer layer about 20–40 μ in thickness, consisting of cubical or somewhat angular cells measuring 15–25 μ in diameter with slightly thickened brown walls, containing large oil globules. Inner layer about 10–20 μ in thickness, consisting of hyaline, thin-walled, strongly compressed cells (Fig. 6).

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Joint contribution from the Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada, and the Botany Department, University of Toronto, Toronto, Ontario. Contribution No. 677 from the Forest Biology Division.

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Paraphyses evanescent, disappearing early. Asci subcylindrical, broadest in middle, slightly narrowed toward each end, (55)61–65(67) × 9.0–13 μ, thin outer wall not rupturing nor swelling extensively in water, sessile, breaking free early to fill the perithecial cavity by gelatinization of stalks (Figs. 6 and 5) with a conspicuous thickened ring in apex, appearing as two elliptical refractive bodies.

Ascospores usually four in each ascus, very occasionally three, rarely five or six, one-celled, hyaline, obliquely or sometimes irregularly uniseriate, (12)13–15(17) × (5)7–9(10) μ, elliptic to strongly inequilateral, with usually two oil globules.

Host: on recently killed terminal twigs of *Alnus* sp. in Ontario (Figs. 1 and 2).

Specimens examined: Canada: Ontario: All on *Alnus* sp. Nansen township, Kapuskasing district,[4] MFB 4962 and 4963; Wacousta township, Cochrane district, MFB 4964; Best township, MFB 4965 and 4966, East Ferris township, MFB 4967 and 4968, and Bastedo township, MFB 4969, all of the North Bay district; Hammer Lake, White River district, MFB 4970; Tweedsmuir township, Kenora district, MFB 4971; Alice township, Pembroke district, MFB 5361; Wickstead township, Kapuskasing district, MFB 5362.

United States. *Alnus* sp. North Greenbush, New York, Univ. of New York State Museum (Type) C. H. Peck.

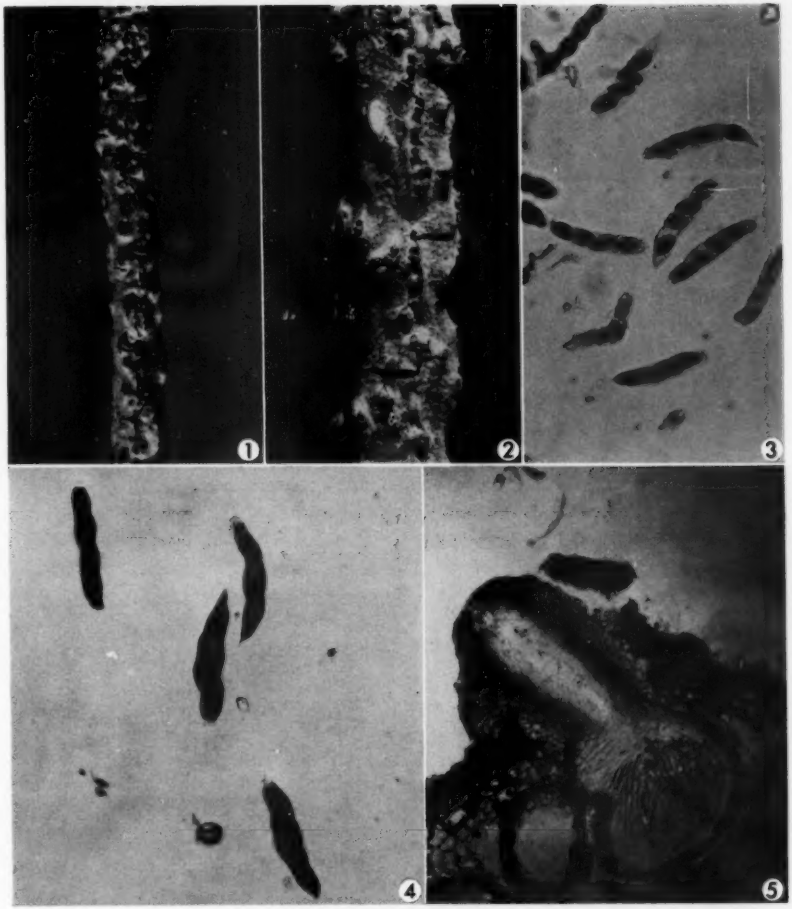
This organism has been collected only rarely in the past. No specimens of this fungus are deposited in the Mycological herbarium, Plant Research Institute, Ottawa, the Farlow herbarium, Harvard University, nor the New York Botanical Garden herbarium. There are, however, three collections in the National Fungus Collections under the name *Cryptospora paucispora* (Pk.) Ell. & Ev. These were all collected from *Salix* sp. on the same date from the same locality near Washington, D.C. Examination, however, revealed they do not agree with the description given above for *C. paucispora*. The only other United States collection of which the writers are aware is the type collection.

Because of the distances between collection points, one would suspect that this species should not be too uncommon in Ontario. On any one host tree, however, there is usually a scarcity of fructifications of the fungus, even though numerous small branchlets are dead on each tree.

The disposition of this organism, and its relationship to other members of the Diaporthaceae is of some interest. According to Wehmeyer[5] this organism belongs in the genus *Cryptosporella* for the following reasons: (1) The perithecia are not effuse but clustered and pustulate erumpent, (2) there is no dorsal nor ventral zone, and (3) there is a light-colored stroma about the perithecia. It does lack the converging ostioles mentioned by Munk (6) and von Arx and Müller (12) in their descriptions of the genus; however, this does not appear to be significant. Peck (7) described this organism as a new species of *Valsa*, although he clearly recognized it as a *Cryptosporella* sp., since the type specimen

[4]The districts referred to are the Forest Districts of the Ontario Department of Lands and Forests.

[5]Personal communication.



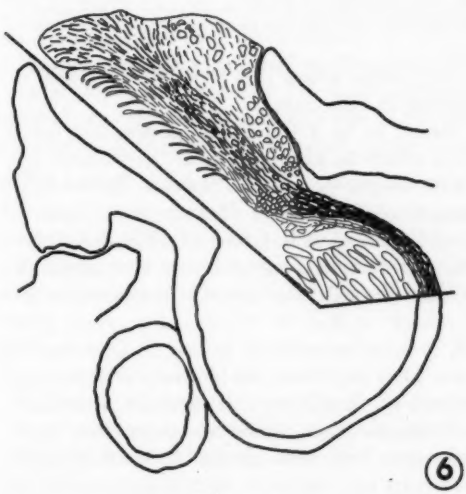
1. Dead terminal twig of *Alnus* sp. bearing mature perithecia of *C. paucispora*. ×3
2. Dead terminal twig of *Alnus* sp. bearing mature perithecia of *C. paucispora*. ×7
3. Asci and ascospores of *C. paucispora*. ×400
4. Asci and ascospores of *C. paucispora* (×500). Note the apical ring at the tip of the asci in both Figs. 3 and 4.
5. Section of mature perithecium of *C. paucispora*. ×100

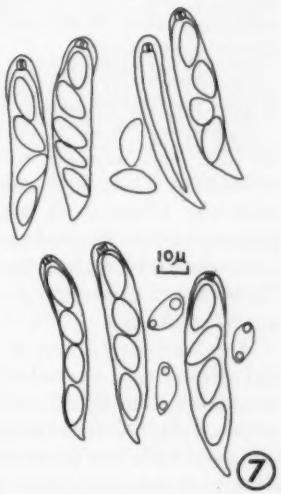
is inserted in the University of New York State Museum herbarium as *Cryptosporella paucispora* Pk., the label being in Peck's own handwriting.

Von Arx and Müller (12) separated the genus *Cryptosporella* from the Diaporthaceae and placed it in a new family, the Cryptosporellaceae of the Sphaeriales. In making this disposition, they have stressed the presence of an apical ascus ring as a basic requirement for an organism to be included in the Diaporthaceae. They have, however, apparently erected this new family on the basis of the nature of the ascus apex in *Cryptosporella hypodermia* (Fr.) Sacc., the only species they have cited, even though they recognized that this family was distinct from all others in the Sphaeriales for the following reasons: (1) the valsoid grouping of the perithecia, (2) the converging ostioles, and (3) the numerous asci whose stalks gelatinized early with the asci then filling the entire perithecial cavity.

This transfer is in contrast to the disposition of this genus made by Munk (6), von Höhnel (11), and Wehmeyer (8), all of whom clearly recognized its true affinity with the Diaporthaceae. Munk (6) states that many genera of the Diaporthaceae in the vicinity of *Cryptosporella* show an atypical development of the apical apparatus and he therefore believes that there is no reason for the erection of a new family.

The structure of *C. paucispora*, and the well-defined apical ring (Figs. 3, 4, and 7) support the view that this genus properly belongs in the Diaporthaceae. It is clearly related to the genus *Gnomoniella*, particularly *Gnomoniella tubaeformis* (Tode) Sacc., due to similarities in spore shape and perithecial structure, differing from it only in the four-spored asci and the presence of a





6. Camera lucida drawing of a section of a mature perithecium of *C. paucispora*. ×160
7. Camera lucida drawing of asci and ascospores of *C. paucispora*. ×600

well-developed clypeus. On the other hand its affinities with other members of the genus *Cryptosporella* serve to properly place the genus in the Diaporthaceae.

A second organism which clearly connects the genus *Cryptosporella* with the Diaporthaceae is *Cryptosporella acerina* L. E. Wehmeyer. While the authors have not examined this organism the description given by Cooke (1) states "Asci numerous, clavate, soon deciduous at the base and free in the perithecium, thin walled with a refractive ring in the apex." This, coupled with other features of the description, clearly indicates that it belongs in the Diaporthaceae, and strengthens the argument against the view held by von Arx and Müller.

Diaporthe taleola (Fr.) Sacc. Fungi Ven. 4: 12. 1875

Sphaeria taleola Fr. Syst. Myc. 2: 391. 1822

For a complete list of synonomy see Wehmeyer (9).

Appearing as small conic pustules with a central disk. Disk white to black, up to 1 mm in diameter. Stroma margined by a black zone, often visible on the surface of the bark. Perithecia 300–400 × 300–400 μ, clustered within the stroma. Ostioles collectively erumpent. Asci elongate cylindric, with an apical ring, 120–160 × 10–14 μ. Spores uniseriate, two-celled, constricted at the septum, with hyaline apical and lateral appendages. Spores 16–29 × 7–12 μ.

Host: *Quercus alba*.

Exsiccati: Krieg, Fung. Sax. 966; Weese, Eum. Sel. Exs. 325 (as *Caudospora taleola*).

Specimens examined: Ontario: MFB 4945, Bathurst Twp., Lanark Co.

The above description was obtained from an examination of MFB 4945, and agrees well with that given by Wehmeyer (9), with the exception that the spores in the Ontario collection are somewhat larger. This is probably not significant since Wehmeyer has noted there is quite a range in the size of spores at maturation.

This species is quite common in Europe, where it is reported by Munk (6) to be a parasite of, and often noxious to, oak trees.

Wehmeyer (9) considered *D. taleola* to be a European species, and transferred all the American collections which he examined to *D. leiphaemia* var. *raveneliana* (De Thüm. and Rehm) Wehm. or to other species. He made no reference, however, to the American collections of Holway from Iowa or Schweinitz from Pennsylvania cited by Ellis and Everhart (2). The description for these species given by Ellis and Everhart agrees with that given by Wehmeyer for European material, although the spore measurements are somewhat smaller.

More recently Gilman *et al.* (4, 5) have recorded *D. taleola* on *Quercus alba* and *Quercus macrocarpa* from Iowa. They examined the Holway collection and considered it to be a good specimen of *D. taleola*. Furthermore, they have collected this organism widely in Iowa, and their figures and description agree so closely with that given by Wehmeyer that there can be no doubt as to the identity of this organism.

This is the first record of a collection of *D. taleola* having been made in Canada.

Diaporthe leiphaemia var. ***raveneliana*** (De Thüm. and Rehm) Wehm. Univ. of Michigan Sci. Ser. IX: 178–179. 1933

Diaporthe raveneliana De Thüm. & Rehm Flora, 61: 178. 1878

Host: *Quercus alba* and *Quercus* sp.

All of the Ontario specimens agree with the description given for the species by Wehmeyer (9). This fungus appears to be a saprophyte or very weakly parasitic on oak trees.

Exsiccati: Ell. and Ev., N. Am. Fungi 93 (as *Valsa leiphaemia*).
Ravenel, Fungi Caroliniani 69 (as *Valsa taleola*).

Specimens examined: Canada: Ontario: London, DAOM 37316 (Dearness 2984); Baxter Twp., Simcoe Co., MFB 5043; Brant Co., MFB 5042; Norfolk Co., TRTC 4317.

United States. TRTC (ex BPI 65, 325), Washington, D.C.: TRTC (ex U. of Tenn. 6159), Montvale Springs, Blount Co.

Apioporthe anomala (Pk.) Höhn. Sitz. Akad. Wiss. Wien, 126: 381. 1917

Diatrype anomala Pk. N.Y. State Mus. Rept. 28: 72. 1876

Cryptosporella anomala (Pk.) Sacc. Syll. 1: 470. 1882

Cryptospora anomala (Pk.) Ell. & Ev. North Am. Pyr. 531. 1892

Host: *Corylus* spp.

Exsiccati: Ell. & Ev., N. Am. Fungi 1185 (as *Diatrype anomala*).
Ell. & Ev., Fungi Columbiani 322 and 731 (as *Cryptospora anomala*).

Specimens examined: Canada: Nova Scotia: Kentville, DAOM 7969. Ontario: Algonquin Park, MFB 5138; Chalk River, MFB 5040; Port Burwell, DAOM 861; London, DAOM 35955 (Dearness 228).

These collections agree with the description given by Wehmeyer (9) although the spores tend to be 1–1.5 μ wider.

A. anomala causes pronounced elongate cankers on the branches of the host. Several cankers on branches $\frac{1}{4}$ to $\frac{1}{2}$ in. in diameter were 14 in. long. Often these cankers are confined to only one side of the branch with the perithecia found distributed along the canker. Later, entire branches of the host tree are killed.

Valsa salicis (Fuckel) Winter. Rabenhorst's Krypt. Flora, 1(2): 745. 1887

Valsella salicis Fuckel. Symbolae Mycologicae: 203. 1869

Stroma circular, up to 1 mm in diameter, pulverulent, dark from above, with a marginal blackened zone, scattered, rarely confluent. Perithecia 6–12 in a stroma, 300–400 μ in diameter, crowded in the stroma. Asci cylindric-oblong, sessile, polysporus, 40–50 $\times$ 6–8 μ. Spores 6–7.5 $\times$ 1–1.5 μ, botuliform, hyaline.

Host: *Salix sp.*

Exsiccati: E. Bartholomew, Fungi Columbiani 3200 (as *Valsella salicis*).

Specimens examined: Ontario: White River district, MFB 5024.

This description agrees with that given by Munk (6) and Winter (10). The exsiccati collection, however, has spores which are shorter, reaching only 6 μ in length, rarely longer.

This organism has not previously been reported from Canada.

Acknowledgments

The authors are grateful to Dr. L. E. Wehmeyer, Professor of Botany, University of Michigan, for the identification of *Cryptosporella paucispora* and for other helpful advice. We also wish to thank Dr. C. R. Benjamin, National Fungus Collections, Beltsville, Maryland; Dr. D. B. O. Savile, Mycological Herbarium, Plant Research Institute, Ottawa; and Mr. S. J. Smith, New York State Museum, University of the State of New York, Albany, New York, for the loan of various specimens.

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NOTES ON SOME GRASSES

X. SOME THOUGHTS ON FESTUCEAE, FESTUCINAE WITH SPECIAL REFERENCE TO THEIR MORPHOLOGY[1]

TUGUO TATEOKA[2]

Abstract

The flowers and caryopses of Festuceae, Festucinae (sensu Pilger, 1954) have been morphologically studied. All the genera, which show non-festucoid features in respect to non-morphological characters, are also different from true Festuceae, Festucinae in such details of their morphology as lodicules, pistils, and caryopses. On the other hand, the festucoid genera are very closely related to one another as regards these organs. The systematic positions of the genera showing non-festucoid traits in non-morphological characters are being considered in connection with their characteristics of flowers and caryopses. The results clearly indicate that the conventional treatment of morphological characteristics, in which stress is laid on some macroscopic characters, such as inflorescences or the appearance of lemmas, is quite inadequate, and that great attention should be paid to the features of flowers and caryopses, as well as spikelets and other morphological characters. A new tribe Brylkinieae is proposed.

Introduction

In the most recent decades, various non-morphological aspects, such as cytology, anatomy, or embryology, have been studied in connection with systematics and helped to clarify relationships of taxa in Gramineae. The increasing number of studies along these new lines of approach has not only revealed some unnatural classification based on more classical traits, e.g., the inflorescences and spikelets, but has also suggested a renewed appreciation of morphological characteristics in systematics.

In 1956, Stebbins reviewed the morphology of two groups of grasses, in which a large number of supposed intergeneric hybrids have been obtained: the genera *Lolium* and *Festuca*, and the tribe Triticeae. In these groups, he shows that, if emphasis is placed upon the features of the flowers themselves and the caryopses, the evidence given by cytogenetic and morphological studies is in good agreement. The importance of lodicules, pistils, and caryopses for grass systematics was also pointed out by Church (1949) and Hubbard (1946, 1948). Church (1949) showed that the differences of lodicules and caryopses existing between *Glyceria* and *Torreyochloa* previously treated as congeneric are parallel to the cytological differences. Hubbard (1946) split the genus *Lepturus* into five genera, and his grouping has been supported by subsequent anatomical and cytological studies (Hansen and Potztal 1954; Tateoka 1959*a*), and the morphological characters which Hubbard uses to separate his genera are for the most part related to flowers and caryopses.

Further understanding of the systematic significance of various morphological characters will be brought forth by studying the interrelationship between morphological and non-morphological characters of members in

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TABLE I

List of the genera of Festuceae, Festucinae (Pilger 1954) whose non-morphological features have been reported

Genera	Chromosomes*	Leaf structure†		Embryo anatomy‡
		Epidermis	Trans section	
Lamarckia	$x=7$, large	Festucoid (Prat)	Festucoid (Prat)	F + F F
Cynosurus	$x=7$, large	Festucoid (Prat)	Festucoid (Prat)	F + F F
Briza	$x=7$, 5, large	Festucoid (Prat)	Festucoid (Prat)	F + F F
Calotheca	$x=7$, large			
Dactylis	$x=7$, large	Festucoid (Prat)	Festucoid (Prat)	F + F F
Poa	$x=7$, medium	Festucoid (Prat)	Festucoid (Prat and Brown)	F + F F
Eremopoa	$x=7$			
Sclerochloa	$x=7$, large			F + F F
Nephelochloa		Festucoid (Prat)	Festucoid (Prat)	
Cutandia	$x=7$, large	Festucoid (Prat)	Festucoid (Prat)	
Desmazeria	$x=7$, large			
Catapodium	$x=7$, large	Festucoid (Prat)	Festucoid (Prat)	
Micropyrum	$x=7$, large			
Nardurus	$x=7$, large			
Vulpia	$x=7$, large	Festucoid (Prat)	Festucoid (Prat)	
Festuca	$x=7$, large	Festucoid (Prat)	Festucoid (Prat and Brown)	F + F F
Pseudobromus	$x=7$, large	Festucoid (de Winter)	Festucoid (de Winter)	(Festucoid)‖
Hesperochloa	$x=7$, large			F + F F
Diarrhena	$x=?$,§ medium	Almost festucoid (Prat and Tateoka)	Festucoid (Tateoka)	F + F F
Brylkinia	$x=10$, medium	Festucoid (Tateoka)	Festucoid (Tateoka)	
Brachyelytrum	$x=11$, small	Intermediate (Tateoka)	Bambusoid (Brown)	F + F P
Plagiochloa	$x=7$, medium–small	Panicoid (de Wet)	Danthonidoid (de Wet)	P − P F
Lasiochloa	$x=7$, medium–small	Panicoid (de Wet)	Danthonidoid (de Wet)	P − P F
Urochlaena	$x=7$, medium–small	Panicoid (de Wet)	Danthonidoid (de Wet)	P − P F
Uniola	$x=12$, small	Panicoid (Prat)	Panicoid (Prat) Bambusoid (Brown)	P + P P
Distichlis	$x=10$, small	Panicoid (Prat)	Panicoid (Prat) Chloridoid (Brown)	P + P F
Aeluropus	$x=10$, small		Type I (Avdulov)	P + P F
Tetrachne	$x=10$, small	Chloridoid (de Wet)	Chloridoid (de Wet)	P + P P
Lintonia	$x=10$, small	Chloridoid (de Wet)	Chloridoid (de Wet)	P + P P
Fingerhuthia	$x=10$, small	Panicoid–chloridoid (Prat) Chloridoid (de Wet)	Chloridoid (de Wet)	P + P P

*Chromosome numbers and sizes indicated in this table are based on a still unpublished list by Tateoka. Most of the investigators of the cytology of the genera appearing in this table have been previously listed in Darlington and Wylie's (1955) Chromosome Atlas. The genera whose chromosome numbers were reported after the publication of Darlington and Wylie (1955) are as follows: *Eremopoa*, Bowden (1960); *Diarrhena*, Tateoka (1957*a*); *Brylkinia*, Tateoka (1957*a*); *Plagiochloa*, de Wet (1960); *Urochlaena*, de Wet (1959); *Fingerhuthia*, de Wet (1960), a.o.; *Lintonia*, de Wet (1960).

†The characteristics of leaf structure were enumerated according to the following papers: Avdulov (1931), Prat (1935, 1936), de Winter (1951), Tateoka (1957*a*, 1957*b*), Brown (1958), de Wet (1959, 1960).

‡The features of embryo anatomy are represented by the letter combination proposed by Reeder (1957). The present table is mostly based on the report by Reeder (1957), with the exception of some South African genera (*Plagiochloa*, *Lasiochloa*, *Urochlaena*, *Tetrachne*, *Lintonia*, and *Fingerhuthia*) which were studied by de Wet (1959, 1960).

‖According to de Winter's (1951) description and figures, the embryo of *Pseudobromus africanus* is characterized by the presence of an epiblast and the lower part of the scutellum is fused to the coleorhiza. These features characterize the festucoid type of embryo anatomy.

§Cf. text.

such a very complicated group as Festuceae, Festucinae. Although the genera of this group are related to one another in gross morphology, they are very diverse with regard to non-morphological characters, showing different types of chromosomes, differences in leaf and embryo anatomy, etc.

Table I lists the genera in Festuceae, Festucinae (sensu Pilger 1954) whose characteristics of chromosomes, leaf structure, and embryo anatomy have been reported. About two-thirds of the genera appearing in Table I display festucoid features in all non-morphological characters so far examined: *Lamarckia*, *Cynosurus*, *Briza*, *Calotheca*, *Dactylis*, *Poa*, *Festuca*, *Eremopoa*, *Sclerochloa*, *Nephelochloa*, *Cutandia*, *Desmazeria*, *Catapodium*, *Micropyrum*, *Nardurus*, *Vulpia*, *Pseudobromus*, and *Hesperochloa*. These genera may represent the main constituents of Festuceae, Festucinae. On the other hand, with respect to chromosomes, leaf structure, and embryo anatomy, *Brachyelytrum*, *Plagiochloa*, *Lasiochloa*, *Urochlaena*, *Uniola*, *Distichlis*, *Aeluropus*, *Tetrachne*, *Fingerhuthia*, and *Lintonia* are different from the true Festuceae, Festucinae. Because of the great differences in the complex of non-morphological characters, these non-festucoid genera ought to be considered as phylogenetically different from Festuceae, Festucinae. *Diarrhena* and *Brylkinia* represent a mixture of festucoid and non-festucoid types of non-morphological characters. The systematic positions of these genera have already been discussed by various authors. The difference, for example, between *Uniola* and other Festuceae has already been pointed out by various authors, e.g., Avdulov (1931), Prat (1936), Reeder (1957), Brown (1958), etc. Brown (1950) mentioned the cytological resemblance of *Brachyelytrum* with Stipeae, when he observed the chromosomes of *B. erectum*. It may be interesting to consider whether the supposed relationship of these genera, as indicated by non-morphological features, can be linked to the features appearing in flowers and caryopses.

The present study aims to disclose the interrelationship between morphological and non-morphological features in some members of Festuceae, Festucinae sensu Pilger (1954), and to help establish the natural grouping of this very complicated group, Festuceae.

Observations

All the observations were carried out on herbarium specimens in the Herbier Marie-Victorin, Institut Botanique de l'Université de Montréal, and in the United States National Herbarium in Washington. The species examined are listed at the end of this paper. Lack of material sometimes prevented detailed studies, but the results described below are considered sufficient to allow a discussion in the following chapter. Since the appearance of the lodicules varies somewhat according to age of the flowers, only florets with a mature caryopsis were used throughout for the observation of lodicules.

1. Festucoid Genera (Figs. 1–12)

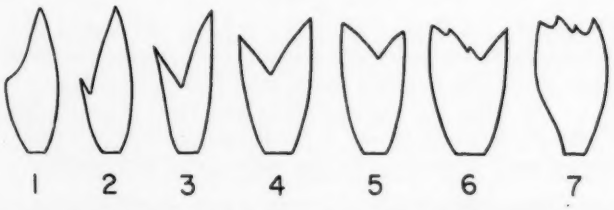
The shape of the lodicules of grasses belonging to the festucoid group varies considerably, but the type shown in Figs. 3–5, in which the lodicule is split

into two asymmetrical and acute halves, is the most common. The form indicated in Fig. 2, in which one of the two lobes is much reduced, is also rather common. In the type seen in Fig. 1 and only rarely found, the small lobe has vanished and the lodicule is entire. The shape shown in Fig. 6, in which one or more lobes are further divided, is observed in some species of *Poa* and *Festuca*. The form indicated in Fig. 7, in which the upper edge of the lodicule is many-toothed, is found in *Sclerochloa dura*. The lodicules of these grasses thus represent a continuous variation from the type of Fig. 1 to that of Fig. 7, centering around the shapes shown in Figs. 3–5. Throughout, the lodicules of these grasses are more or less elongated, usually glabrous, rarely with a few hairs at the upper edge, and show no distinct vascular trace. It should be noted, however, that the presence of vascular traces is sometimes obscure when superficially observed under a binocular as was done in the present study, and it seems desirable to study this question further from an anatomical point of view. According to Reeder and Ellington (1960), another feature of festucoid lodicules, which is very evident at anthesis, is the rounded cushion at the base, and the thin membranous upper part.

The ovary of the grasses in this group is mostly glabrous, but hairy in *Hesperochloa*, *Pseudobromus*, and some species of *Festuca*. The styles number two, are usually obscure, and the stigma branches directly from or somewhat above the tip of the ovary; none of the taxa has long and very distinct styles. The caryopsis is usually elliptical, oblong or ovate, and bears ordinarily a small or large membranous appendage at the tip. In species whose ovary is hairy the apex of the caryopsis is also hairy. Often a stigma remnant is found at the tip. The embryos are small, and do not reach one-third the length of the caryopsis and very rarely become somewhat longer than it. The shape of the hilum varies from punctiform to linear.

2. Plagiochloa (Figs. 13–18)

This genus has two lodicules, which are cuneate or oblong, fleshy, have few or many, long or short, hairs on the upper edge, and show several vascular traces. The pistils were studied only in *P. uniolae*. Its ovary is glabrous, and two short styles arise from the tip of the ovary. Material for observation of the caryopsis was obtained from two species, *P. acutiflora* and *P. brachystachys*. In both, the caryopsis is oblong-oval and bears a membranous ap-



FIGS. 1–7. Shapes of lodicules of festucoid genera: 1, *Cutandia maritima*; 2, *Briza minor*; 3, *Vulpia myuros*; 4, *Poa grayana*; 5, *Festuca idahoensis*; 6, *Poa gracillima*; 7, *Sclerochloa dura*.

pendage on the tip. The embryos are comparatively large, showing a length ratio of one-third to one-half of the caryopsis. The hilum is round and basal.

3. Urochlaena

No material was available, but, according to de Wet (1959), the lodicules of this genus are cuneate.

4. Lasiochloa (Figs. 19–20)

The lodicules are two, cuneate, fleshy, with a few vascular traces and a few, long or short, hairs on the upper edge, rarely hairless. Pistils and caryopses could not be observed.

5. Brylkinia (Figs. 29–30)

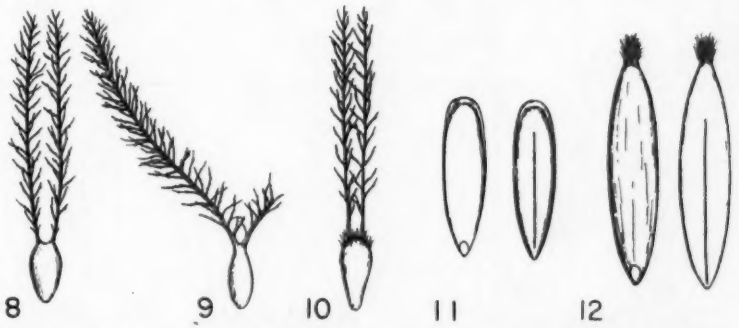
The two lodicules are large and broad, rectangular, membranous, and without distinct vascular traces. The ovary is glabrous, the tip pointed like a beak, from which two styles arise. The caryopsis is glabrous with a hardened and glossy cap. The embryo is about one-fifth the length of the caryopsis.

6. Diarrhena (Figs. 31–35)

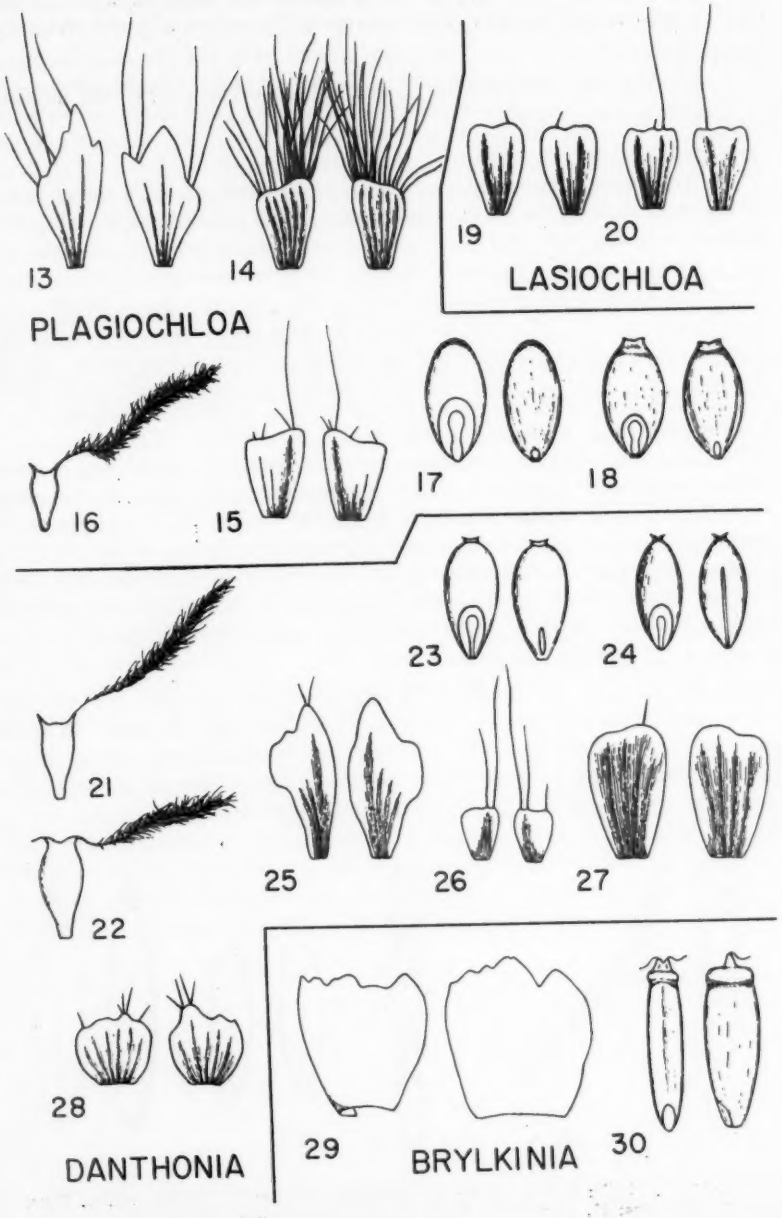
The two lodicules are lanceolate or two-toothed, membranous, brown-colored around the center with a fold, and have fine cilia-like hairs at the tip. The ovaries are glabrous, and have two short styles. The caryopsis of *D. americana* has a large, hardened, glossy, and yellowish cap at the tip. This peculiar appendage is also recognizable in pistils and immature caryopses. In *D. japonica*, the caryopsis appendage is smaller and nipple-like, and the embryo is about one-third the length of the caryopsis.

7. Brachyelytrum (Figs. 36–39)

The two lodicules are elongated, oblong, membranous, with a few vascular traces, and a fold at the base. The ovary has a peculiar character, its upper part having many bristles. The part with the bristles develops into an appendage like a beak in the mature caryopsis. Two styles are distinct. The caryopsis is linear, with a small embryo and a linear hilum.



FIGS. 8–10. Pistils: 8, *Poa reflexa*; 9, *Cynosurus cristatus*; 10, *Festuca obtusa*. FIGS. 11–12. Caryopses: 11, *Micropyrum tenellum*; 12, *Pseudobromus africanus*.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BRYLKINIA
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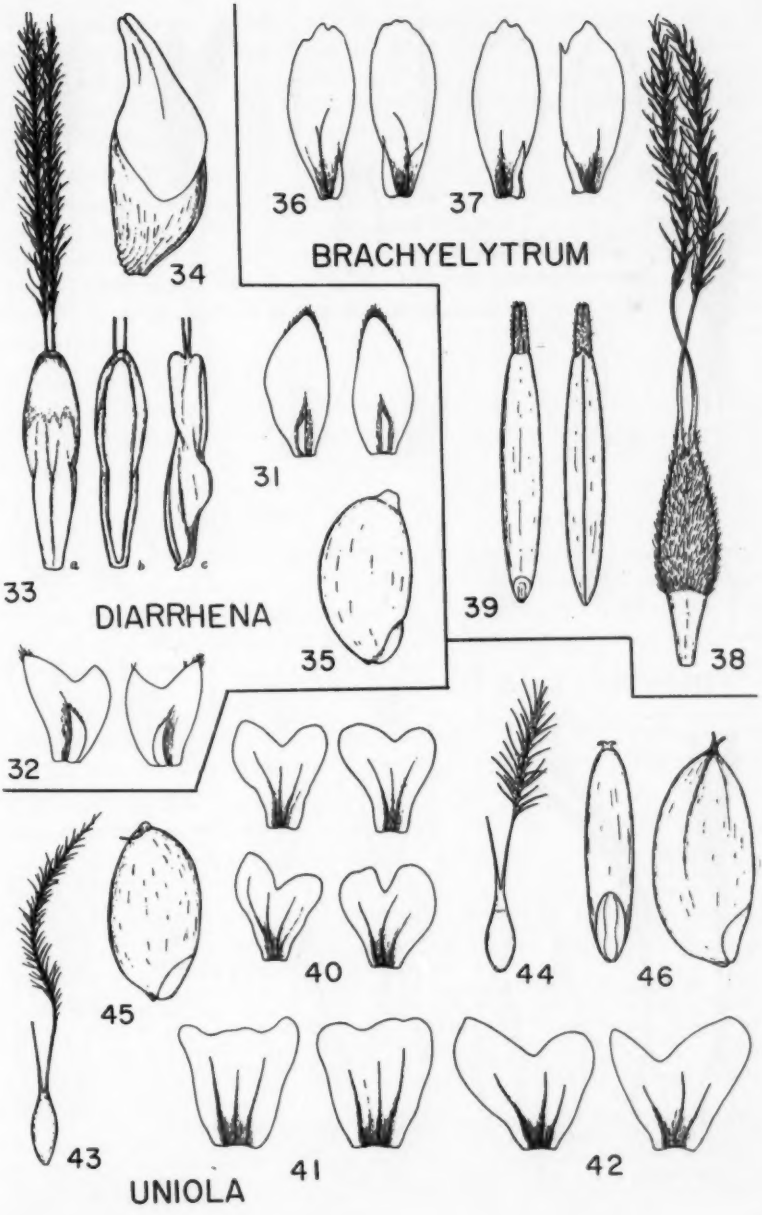
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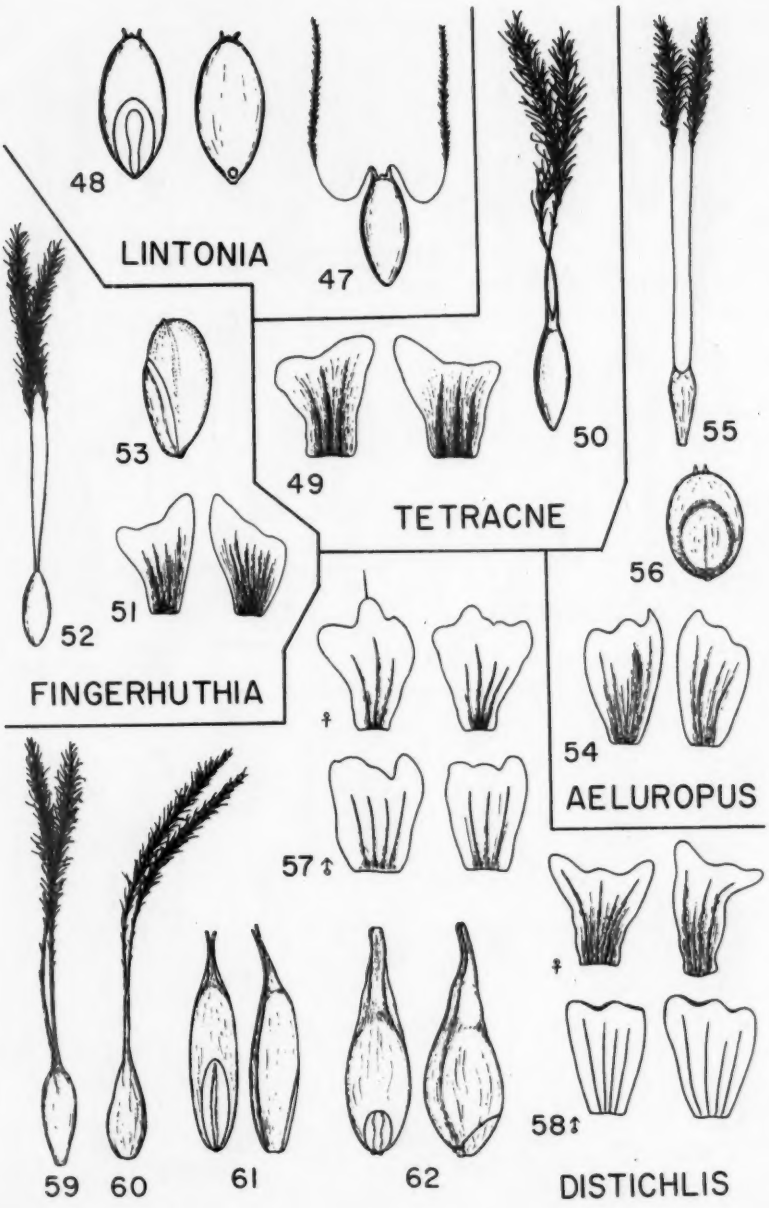
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FINGERHUTHIA
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AELUROPUS
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DISTICHLIS

8. Uniola (Figs. 40–46)

This genus has two lodicules, which are cuneate or heart-shaped, usually membranous, and have a few vascular traces. Two distinct styles arise from the tip of the ovary. The caryopsis is hairless, compressed laterally, oblong-ovate, its tip showing a small and hardened appendage. The embryos are about one-third the length of the caryopsis.

9. Distichlis (Figs. 57–62)

The lodicules are two, cuneate, and have several vascular traces; their upper edge is more or less dentate, more or less fleshy in young flowers and in male florets, and rather membranous in pistillate florets with a mature caryopsis. Very rarely they carry a few hairs on the upper edge. Embryos are small, or occasionally rather large, sometimes reaching half the length of the caryopsis.

10. Aeluropus (Figs. 54–56)

Its two lodicules are obovate or cuneate, with a few vascular traces. The ovary is glabrous. The two styles are long and very distinct. The caryopsis is roundish and has remnants of styles at the tip. The embryo is large, about two-thirds of the caryopsis in length.

11. Lintonia (Figs. 47–48)

The two lodicules are elongate, rectangular, fleshy, and have several vascular traces. The ovary is glabrous. The two styles are long and very distinct with dark-purple stigmas. The caryopsis is ovate-oblong with style remnants at the tip. The embryo is about half the length of the caryopsis. The basal hilum is punctiform.

12. Tetrachne (Figs. 49–50)

This genus has two cuneate and fleshy lodicules with a few vascular traces. Its ovary is glabrous. The two styles are long and very distinct. The caryopsis could not be observed.

FIGS. 13–18. *Plagiochloa*: 13–15, lodicules (13, *P. brachystachya*; 14, *P. uniolae*; 15, *P. ciliaris*); 16, pistil of *P. uniolae*; 17–18, caryopses (17, *P. aculiflora*; 18, *P. brachystachya*). FIGS. 19–20. Lodicules of *Lasiochloa* (19. *L. longifolia*. 20, *L. obtusiflora*). FIGS. 21–28. *Danthonia*: 21–22, pistils (21, *D. parryi*; 22, *D. californica*); 23–24, caryopses (23, *D. spicata*; 24, *D. californica*); 25–28, lodicules (25, *D. californica*; 26, *D. curva*; 27, *D. parryi*; 28, *D. unispicata*). FIGS. 29–30. *Brylkinia caudata*: 29, lodicules; 30, caryopses (not completely mature). Right, front view. Left, side view.

FIGS. 31–35. *Diarrhena*: 31–32, lodicules (31, *D. americana*; 32, *D. japonica*); 33, pistil of *D. americana*. (*a*, front view; *b*, back view; *c*, side view); 34–35, caryopses (34, *D. americana*; 35, *D. japonica*). FIGS. 36–39. *Brachyelytrum*: 36–37, lodicules (36, *B. erectum*; 37, *B. japonicum*); 38, pistil of *B. erectum*; 39, caryopsis of *B. erectum*. FIGS. 40–46. *Uniola*: 40–42, lodicules (40, *U. laxa* (two florets); 41, *U. latifolia*; 42, *U. sessiliflora*); 43–44, pistils (43, *U. latifolia*; 44, *U. laxa*); 45–46, caryopses (45, *U. latifolia*; 46, *U. laxa*). Right, front view. Left, side view.

FIGS. 47–48. *Lintonia nutans*: 47, pistil; 48, caryopsis. FIGS 49–50. *Tetrachne dregei*: 49, lodicules; 50, pistil. FIGS. 51–53. *Fingerhuthia*: 51, lodicules of *F. sesleriaeformis*; 52, pistil of *F. africana*; 53, caryopsis of *F. africana*. FIGS. 54–56. *Aeluropus littoralis*: 54, lodicules; 55, pistil; 56, caryopsis. FIGS. 57–62. *Distichlis*: 57–58, lodicules (57, *D. stricta*; 58, *D. palmeri*); 59–60, pistils (59, *D. spicata*; 60, *D. stricta*); 61–62, caryopses (right, front view; left, side view; 61, *D. palmeri*; 62, *D. stricta*).

13. Fingerhuthia (Figs. 51–53)

The lodicules of this genus were observed in *F. sesleriae-formis*. They are cuneate, fleshy, and have a few vascular traces. Pistils and caryopses were examined in *F. africanus*. Its ovary is glabrous, the two styles are long and distinct, and the caryopsis glabrous, somewhat compressed laterally. The embryo is large, about two-thirds the length of the caryopsis.

Discussion

From the above-mentioned descriptions and figures, it may be derived that the genera which show differences from true Festuceae, Festucinae grasses in non-morphological characters are also different in the appearance of their lodicules, pistils, and caryopses. On the other hand, the genera having festucoid chromosomes, leaf structure, and embryo anatomy are very closely related to one another also in respect to their flowers and caryopses. The systematic position of the genera dealt with in the present study has already been discussed by various authors. Some remarks will be given below regarding the genera showing differences from true Festuceae, Festucinae in non-morphological characters connected with their features in flowers and caryopses as described above.

Brachyelytrum is similar to Festuceae, Festucinae, in such features as the glumes being shorter than the lemma, the latter having five distinct nerves, etc. However, the lodicules, pistils, and caryopses of *Brachyelytrum* are apparently very different from true Festuceae, Festucinae. The lodicules of this genus are elliptical and have some vascular traces and a fold at the base. The upper part of its pistil has many bristles which develop into a peculiar appendage like a beak when the mature caryopsis is formed. Such characters are never found in true Festuceae, Festucinae. Based on some distinctive features of spikelets the formation of a separate tribe, Brachyelytreae, was proposed by Ohwi (1941), and this view was taken up by Tateoka (1957*b*), who discussed the systematic position of the genus from cytological, anatomical, and morphological standpoints. The separate position of *Brachyelytrum*, suggested already by the combination of its non-morphological traits, is also clearly indicated by the peculiarities of its flower and caryopsis.

De Wet (1959, 1960), basing his ideas on the examinations of various characters of significance in grass systematics, has transferred the genera *Plagiochloa*, *Lasiochloa*, and *Urochlaena* to Danthonieae. In Figs. 21–28, the lodicules, pistils, and caryopses of some species of *Danthonia* are illustrated. It is clear from these figures (Figs. 13–28) that *Plagiochloa*, *Lasiochloa*, and *Urochlaena* are closely related to *Danthonia* in respect to these organs. In both groups, the lodicules are cuneate or elongated, and have several distinct vascular traces and long or short hairs on the upper edge. The ovaries of these genera are glabrous, and the styles are short. The caryopses have a comparatively large embryo. On the other hand, they are clearly different from true Festuceae, Festucinae not only in various non-morphological characters but also in flowers and caryopses. In true Festuceae, Festucinae, the lodi-

cules are mostly split into two asymmetrical halves, though the shapes vary considerably (Figs. 1–7). Furthermore they are membranous, and have no distinct vascular traces. The caryopsis has a small embryo. The relationship of *Plagiochloa*, *Lasiochloa*, and *Urochlaena* to Danthonieae, which was already suggested from the resemblance in non-morphological characters, is thus clearly reflected also in morphological features, although not in such characters which are customarily considered as important.

In the case of *Uniola*, there are also very apparent differences with respect to lodicules, pistils, and caryopses from those of true Festuceae, Festucinae. The establishment of the tribe Unioleae, which has already appeared in various publications, seems to be justified by the distinct morphological and non-morphological features found in the genus.

Distichlis has eragrostoid features in type of chromosomes, embryo, and leaf anatomy. In the traits of its epidermis, the genus is somewhat different from the main constituents of Eragrostoideae, but yet there exists a great resemblance. The lodicules of *Distichlis*, which are cuneate and more or less fleshy, are very similar to those of eragrostoid grasses. The lodicules, pistils, and caryopses of *Aeluropus* also suggest its affinity to Eragrostoideae. The styles of *Aeluropus*, as well as those of *Tetrachne*, *Fingerhuthia*, and *Lintonia*, are distinct. Such distinct styles can not be found in true Festuceae, Festucinae, but are common in Eragrostoideae. Also, in eragrostoid grasses the embryos are large and the lodicules are usually cuneate, similar to those found in *Aeluropus*, *Tetrachne*, *Fingerhuthia*, and *Lintonia*. Therefore, the separation of these genera from Festuceae as well as their affinity to eragrostoid grasses are apparently supported by the morphological features when emphasis is placed upon the flowers and caryopses. This conclusion is in conformity with the evidence shown in non-morphological characters.

Brylkinia, a monotypic genus found in East Asia, is usually considered a relative of *Uniola* (Bentham and Hooker 1883; Hackel 1887; Bews 1929; Pilger 1954; Stebbins 1956*b*), as in both genera a few lower florets are reduced and the spikelets are laterally compressed and keeled. However, conspicuous differences are found between them not only in various non-morphological features, but also in details of the flowers and caryopses, as well as in some other morphological characters. These details are listed in Table II. The embryo anatomy of *Brylkinia* has not yet been examined, but in nearly all other diagnostic characters, *Brylkinia* is definitely unlike *Uniola*. The resemblance between them apparently lies only in gross morphology.

Another supposed relationship of *Brylkinia*, i.e., the inclusion of the genus into Meliceae (s. lat.) as a separate subtribe, has been presented by Ohwi (1942). His reasoning is based upon some resemblance in gross morphology: the inflorescence of *Brylkinia* is a raceme showing some similarity to that of certain members of *Glyceria*, and further, the margins of its leaf sheath are connate and its lemmas are many-nerved. *Brylkinia* fits better here, as this genus and the glyceriiform grasses are also similar in chromosomes, leaf structure, and the organization of starch grains. However, conspicuous

TABLE II

Morphological and non-morphological differences between *Uniola* and *Brylkinia*

	Uniola	*Brylkinia*
Basic number of chromosomes	$x=12$	$x=10$
Trans-sections of leaves	Panicoid (according to Prat 1936)	Festucoid
Leaf epidermis	Panicoid	Festucoid
Starch grains of seeds	Simple or compound	Compound, each grain being composed of many granules
Lodicules	Cuneate or heart-shaped, with distinct vascular traces	Rectangular, broad and large, membranous, without distinct vascular traces
Caryopsis apex	With a hardened and small appendage	With a large, glossy and hardened cap
Spikelet disarticulation	Rachilla disarticulates above the glumes and between the florets	Rachilla does not disarticulate, the uppermost fertile floret falling with the pedicel, glumes, and two lower lemmas
Inflorescence	Panicle	Raceme
Margins of leaf sheath	Free	Connate

differences between them are found in the characteristics of flowers and caryopses, and also in floret arrangement, disarticulation of spikelets, and bracts. The lodicules of Meliceae are usually fleshy and often connate, but those of *Brylkinia* are membranous and free. Apparent differences between the two groups are also found in the pistils and caryopses. The styles of Meliceae are distinct, and a pistil with the tip like a beak and the peculiar cap of the caryopsis apex as found in *Brylkinia* are never met with in any glyceriiform grasses. In *Brylkinia*, the spikelets are strongly compressed laterally, the glumes are similar to the lemmas in texture, the two lower florets are reduced and lack a palea and a flower, and the uppermost (third) fertile floret falls off with the two lower sterile lemmas, the glumes, and a pedicel. In Meliceae, the spikelets are usually convex on the back, the reduction of florets occurs in the upper florets and not in the lower ones, and the rachilla disarticulates above the glumes and between the florets (in some species of *Melica*, the entire spikelets fall).

Brylkinia appears to be worthy of distinction as an independent tribe, Brylkinieae,[3] because the genus seems not to fit in any other tribe so far proposed. The new tribe should be placed in Festucoideae, provided that this

[3]Brylkinieae Tateoka, trib. nov.
Festuceae–Brylkiniinae Ohwi in Gramina Japonica I, Acta Phytotax. Geobot. 10, p. 107 (1941).
Spiculis majusculis valde compressis, pedicello brevi pendentibus rachilla ultra florem producta, glumis 5 acute carinatis glabris chartaceis. Gen. *Brylkinia* Fr. Schm. Chromosome number of its single component, *B. caudata* Fr. Schm. is $2n = 40$ (Tateoka 1957*a*).

subfamily also includes Meliceae, Nardeae, Stipeae, etc., together with such festuciform grasses as are represented by Festuceae, Aveneae, Monermeae, and even Triticeae.

Diarrhena, which comprises four to five species, is festucoid in embryo anatomy. In leaf structure the genus is almost festucoid, but Prat (1935) and Tateoka (1957*a*) noted that it deviates somewhat from the typical festucoid characters by certain features of the epidermis. The chromosomes of *Diarrhena* are different from those of the true Festuceae, Festucinae, indicating probably a different basic number and being smaller in size. *D. americana* has $n = 30$ (E. D. Anderson, unpublished, No. 1048, ISC), but the two Japanese species, *D. japonica* and *D. fauriei*, have $2n = 38$ (Tateoka 1957*a*). The basic number deduced from the American species may be 10, and the number $2n = 38$ found in Japanese species could be derived through the loss of two chromosomes from its tetraploid number, $2n = 40$. This supposition seems to be tenable, but the basic number of *Diarrhena* can not be yet firmly decided. Nevertheless, it seems that neither the numbers $2n = 60$ nor $2n = 38$ can be derived from the festucoid type characterized by $x = 7$ and large chromosomes. In correlation with these cytological differences, a study of the morphology also shows that the pistils and caryopses of *Diarrhena* are quite different from those of Festuceae, Festucinae, as indicated in Figs. 8–12 and 33–35. The caryopsis of *D. americana* bears a hardened, glossy, and yellowish appendage, but in the East Asiatic species the appendage is smaller and nipple-like. The lodicules of *Diarrhena* are not so different from those of Festuceae, but the characteristics of the former are never concomitant with those typical of Festuceae, Festucinae. As far as the observations go, the fine cilia-like hairs found on the upper part of *Diarrhena* lodicules have not been found on the lodicules of Festuceae, Festucinae. It should be mentioned here that the genus *Diarrhena* seems to be differentiated into two groups, an American and an East Asiatic one, because of their different types of chromosomes and morphology. The author is rather inclined to believe that the establishment of a separate tribe, Diarrheneae, may be the best way to arrange *Diarrhena* into the grass system, but because further studies seem to be desirable he will confine himself here only to pointing out its peculiarities.

All cases described above clearly demonstrate that the evidences of nonmorphological characters agree with the facts obtained from the studies of flowers and caryopses. Although, in classifying grasses, attention should apparently be paid to all the characters of flowers and caryopses, as well as spikelets and other morphological characters, the lodicule, pistil, and caryopsis will be briefly considered below, because the importance of these organs has been only scantily acknowledged so far.

In 1909, Krause admitted the systematic importance of lodicule features. But, it is only in recent time that the value of this organ has become generally accepted by the investigators of grasses. After Stebbins (1956*b*) enumerated the lodicule as one of the best diagnostic characters in grass systematics, this organ has been discussed by some authors (Reeder and Ellington 1960;

Tateoka 1959*b*). However, an extensive survey of the lodicules in all the groups of Gramineae still seems lacking.

Styles of grass flowers are usually considered of less significance in systematics. The result of the present study, however, indicates that this character really has some systematic value. In true Festuceae, Festucinae styles are usually obscure, but in *Distichlis*, *Aeluropus*, *Fingerhuthia*, *Tetrachne*, and *Lintonia*, they are as long and distinct as those found in many of the Eragrostoideae. Attention should also be paid to the appendage of pistil and caryopsis. In the case of *Brachyelytrum*, its phylogenetic distinction from Festuceae, which is sustained also by non-morphological traits, is most strongly reflected in the differences found in the pistils and caryopses. The caryopsis appendage of *Uniola* also differs from that of true Festuceae, Festucinae, the latter usually having a small or large membranous appendage at the caryopsis apex.

Martin (1946) presented drawings of transverse and longitudinal sections of the caryopsis of 91 grass genera. According to his drawings, most festucoid genera have an embryo that is small in relation to the caryopsis, although in some genera, such as *Alopecurus*, *Briza*, *Phleum*, and *Deschampsia*, the embryos are comparatively large but yet rarely attain half the length of the caryopsis. In eragrostoid and panicoid genera, the embryos are large, at least one-third the length of the caryopsis and usually half its size or more. The significance of this character in grass systematics has been discussed by Reeder (1957) on the basis of his ample experience with grass caryopses. Regarding the genera treated in the present study, the same conclusions as Reeder drew are obtained: *Lintonia*, *Fingerhuthia*, and *Aeluropus* have embryos larger than one-half of the caryopsis, whereas the embryos of true Festuceae, Festucinae are usually less than one-third of the caryopsis in length.

In conclusion, the result of the present study very clearly supports Stebbins's (1956*a*) view that "The morphological characteristics which reveal more consistently the true interrelationships between species and genera must therefore be sought in microscopic characters of the vegetative organs, such as the leaf epidermis and anatomy, and particularly in the structures of the flowers themselves, such as lodicules, styles and stigmas, and caryopsis" (p. 241). If morphological characters are used according to conventional treatment, cases of contradictions will often arise when morphological traits do not coincide with those of non-morphological characters. When, however, we treat the morphological characters in a revised sense, in which emphasis is placed upon the flowers and caryopses, the evidences indicated by both morphological and non-morphological characters can be very reasonably interpreted in a logical manner.

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LIFE HISTORY STUDIES OF THE PACIFIC COAST MARINE ALGA, COLLINSIELLA TUBERCULATA SETCHELL AND GARDNER[1]

ROBERT F. SCAGEL

Abstract

Culture studies of a marine green alga, described from the Pacific Coast of North America as *Collinsiella tuberculata* Setchell and Gardner, have shown it to be a stage in the life history of a form of *Enteromorpha intestinalis* (L.) Link. The *Collinsiella*-stage is thought to be the sexual phase, or a basal remnant of the *Enteromorpha*-stage, and produces biflagellate gametes. The typical tubular thallus of *Enteromorpha* has been grown in culture from zygotes of the *Collinsiella*-stage and zoospores of the *Enteromorpha*-stage. Parthenogenetic development of gametes is also indicated. Some of the tubular plants produce biflagellate gametes which cannot be distinguished from those of the *Collinsiella*-stage. Others produce quadriflagellate zoospores, which may be meiospores or mitospores. An accessory method of reproduction by quadriflagellate mitospores (neutral spores) occurs and this asexual cycle has been carried through three successive generations in culture without an intervening sexual phase. Tubular thalli of *Enteromorpha intestinalis* (L.) Link producing biflagellate gametes during the spring and early summer and quadriflagellate zoospores during the late summer are found in nature in the same area from which the *Collinsiella*-stage was obtained. These are believed to represent in nature the normal sexual life cycle of this plant, which has been reported to have an alternation of isomorphic generations. The possible relationships between the stages obtained in culture and those observed in nature are discussed. A number of hypothetical conclusions have been drawn with respect to the systematic position of the genus *Enteromorpha* and related genera. These studies are being continued to clarify several unknown aspects brought out by this preliminary study. A cytological study is being undertaken to substantiate the conclusions drawn from the morphological data.

Introduction

A small marine green alga first described in 1903 as *Collinsiella tuberculata* S. and G. by Setchell and Gardner (26) from a collection made by Gardner (NLG 403) from the west coast of Whidbey Island, Washington, has been little known until quite recently. *Collinsiella tuberculata*, the type of the genus, is the only species known from the Pacific coast of North America and is also recorded from Japan (37, 25, 9). Two additional species (*C. cava* (Yendo) Printz and *C. japonica* (Yendo) Printz), also recorded from Japan (37, 22, 25, 9) were first described in 1903 by Yendo, who placed them in the genus *Ecballocystis*.

The author first encountered *Collinsiella tuberculata* on July 31, 1958, in Haro Strait, on the west side of San Juan Island, Washington, just south of Deadman Bay (RFS 393). Its resemblance to the basal region of mature plants of the Ulvaceae prompted a study of the plant in culture.

Taxonomy and Distribution

The type of *Collinsiella tuberculata* S. and G. is in the Herbarium of the University of California, Berkeley (UC 97694). Duplicates from the type locality were distributed by Setchell and Gardner among their "Algae of

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Contribution from the Institute of Oceanography, University of British Columbia, Vancouver 8, B. C.

Puget Sound" series, and also by Collins, Holden, and Setchell in their Phycotheca Boreali-Americana (12, Fasc. XIX, No. 909). These specimens apparently all came from a single locality on the west coast of Whidbey Island and were collected on June 6, 1901 (UC 807914). A few specimens are also recorded from the Farallon Islands, near San Francisco, California (26), and from Point Carmel, California (27). At about the same time (1903), Yendo (37) found a plant at Port Renfrew, on the west coast of Vancouver Island, British Columbia, which was referred to the genus *Ecballocystis* and named by him as *E. willeana* Yendo. Yendo had sent his material to Wille, who suggested it should be described as a new species of *Ecballocystis*, a genus formerly known only from fresh water, and both of these authors (37, 32) considered *Collinsiella tuberculata* to be a young and sterile plant closely related to *E. willeana*, if not the same species. This same entity, collected by Tilden on August 4, 1898, at Port Renfrew, was distributed in 1903 (30, Century VII, Fasc. 1, No. 621) as *E. willeana* Yendo. Collins (10, 11), however, considered Yendo's plant from Vancouver Island to be a synonym of *Collinsiella tuberculata*, and West (31) excluded it from the genus *Ecballocystis*. The opinions of Collins and West were subsequently held to the present time by most phycologists on the Pacific coast of North America (27, 28, 13, 24), as well as in Japan (25, 9). The only other published record on our Coast is that of Smith (28) from Point Aulon, near Carmel, California. It is known also from a herbarium specimen (UC 571406) collected and identified by Gardner in 1910 from Waldron Island, Washington.

On comparison with the herbarium material the plants collected on San Juan Island were found to agree morphologically and anatomically with the descriptions and voucher specimens of *Collinsiella tuberculata* referred to above. The type as well as the exsiccatae material distributed in the Phycotheca Boreali-Americana were found to be fertile, just as described by Setchell and Gardner (26, 27), and in this respect the living material from San Juan Island also agreed with the herbarium specimens and descriptions. The plants distributed by Tilden and the material from Waldron Island were both apparently sterile.

Morphology and Reproduction of *Collinsiella*

Setchell and Gardner (26, 27) and Yendo (37) gave detailed descriptions of the structure of *Collinsiella tuberculata*. But they had apparently studied the plant only from preserved material and its reproduction was not clearly established by them. These authors had all noted that the protoplasts of many of the terminal cells of the branched filamentous system (or surface cells)

FIGS. 1–7. The *Collinsiella*-stage.
1. Three groups of mature plants.
2. Surface cells (gametangia) with daughter protoplasts (×500).
3. Biflagellate gametes under dark field illumination (×500).
4. Cells near the surface, embedded in matrix, showing immature gametangia; vegetative cells showing a single large pyrenoid (stained with IKI) (×500).
5. Cells near the surface, embedded in matrix, showing mature gametangia; vegetative cells showing a single large pyrenoid (stained with IKI) (×500).
6. Vegetative cells of the thallus embedded in extensive matrix.
7. Surface close-up view of mature plant (×8).

PLATE I

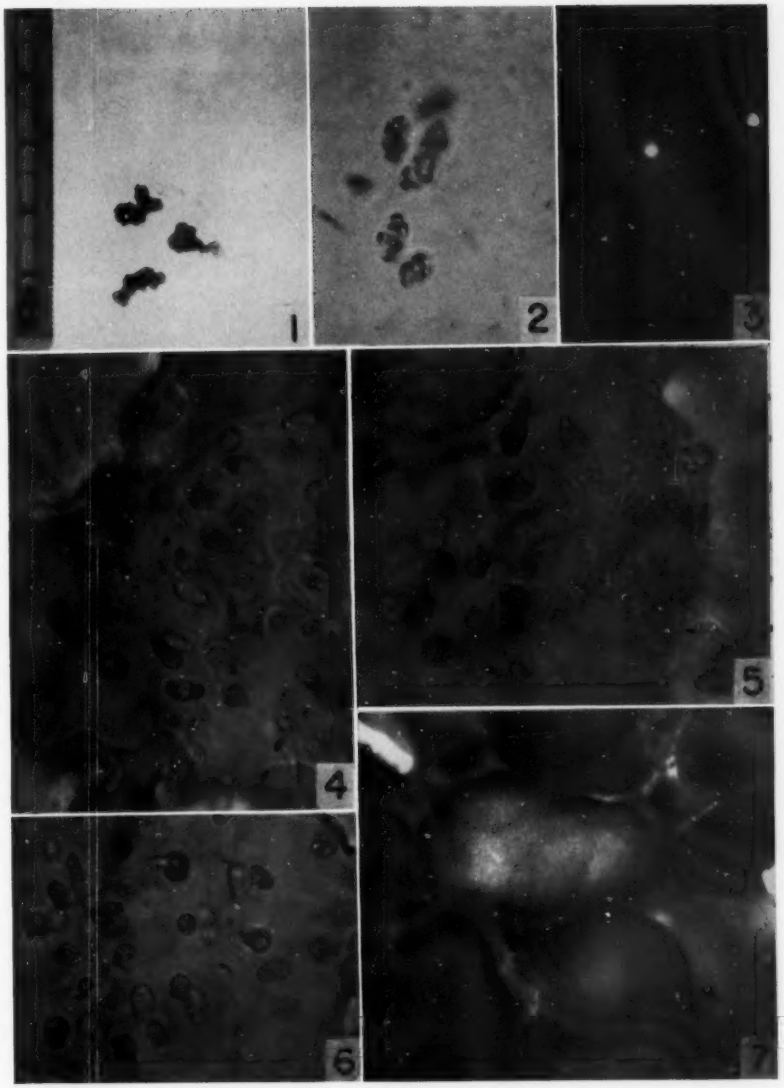
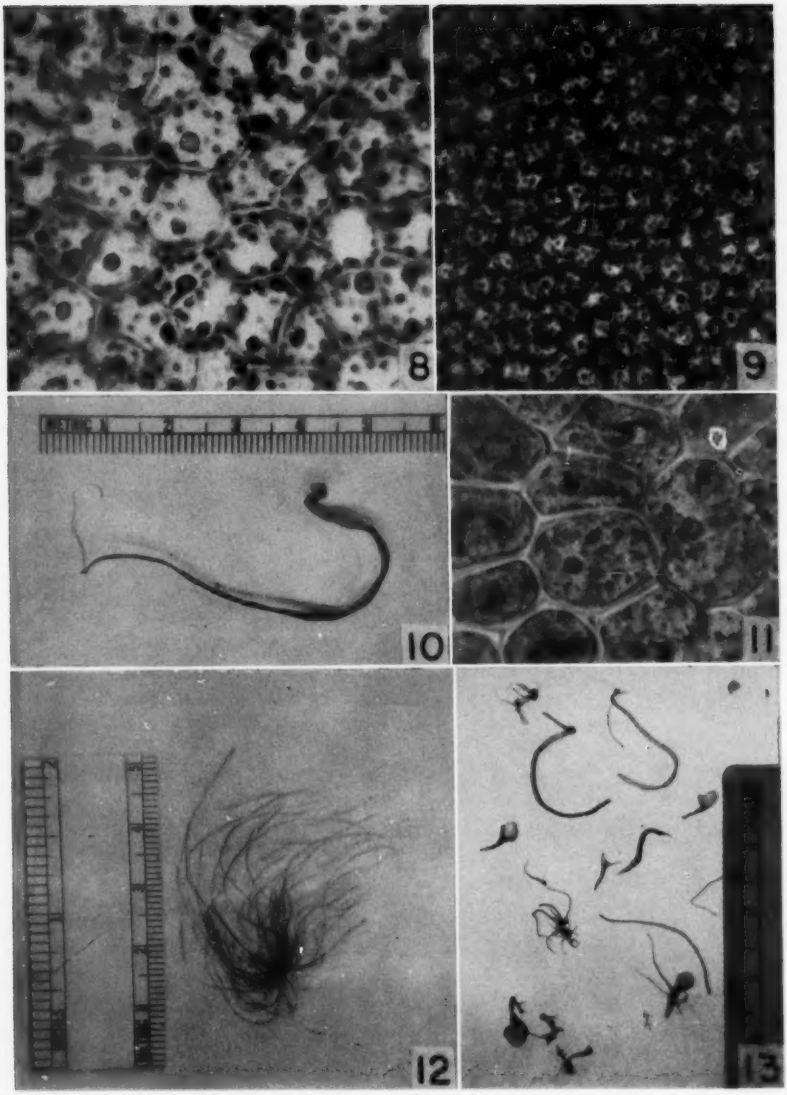


PLATE II



making up the "colonial" thallus were frequently several times divided. Setchell and Gardner (27) considered these surface cells to be "zoosporangia (?)" and Yendo (37) referred to them as "zoosporangia (gametangia?)". None of these authors had apparently observed liberation of the daughter protoplasts found within these "reproductive" cells.

Gross Morphology

Although the plants may occur singly as globose individuals measuring 2–4 mm in diameter, most of them as observed on San Juan Island during this study were growing fused together (Figs. 1 and 7) in dense aggregations on the surfaces of smooth boulders in the middle intertidal zone. They form a compact, dark green, firm, tuberculate covering and the surface of this encrusting layer is quite slippery when wet.

Anatomy

The cells making up the "colony" are arranged in a repeatedly branched system of mucilaginous "stalks" which radiate out through a homogeneous mucilaginous matrix (Figs. 4, 5, and 6). In the vegetative condition the cells are pyriform in shape tapering toward the proximal end and those at or near the surface measure 5–12 μ broad and 12–20 μ long (Figs. 4 and 6). In the vegetative condition the cells contain a single laminate chromatophore, situated in the distal end of the cell, with a single large pyrenoid (Fig. 6). The plants collected in July and August on San Juan Island were fertile (Figs. 4 and 5).

Culture Material

Some of the plants collected in July and August were washed thoroughly and cultures were established in the laboratory. In addition to the plants collected in July, 1958, others were obtained from the same area on August 15 of the same year and again the following year on July 4, 1959, and additional cultures were started from both of these collections. The living plants obtained both summers (1958 and 1959) were kept under observation in the laboratory at the Friday Harbor Laboratories of the University of Washington throughout the summer. Later they were transferred to a constant environment room at the University of British Columbia where they have been in continuous cultivation until the present time. All of the plants studied in culture have been grown in sterilized and enriched sea water.

In the constant environment room the algae were grown at a temperature of 12° C under cool, white fluorescent lights at an intensity of 200–300 ft-c and under a 12/12 hour light/dark schedule of illumination. The cultures

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FIGS. 8–13. The *Enteromorpha*-stage.

8 and 9. Surface view showing angular cells without any definite arrangement; single large pyrenoid and starch grains visible after staining with IKI (Fig. 8 ×1000; Fig. 9 ×350).

10. Plant grown in culture from which mitospores (neutral spores) were obtained.

11. Surface view showing uninucleate cells (stained with acetocarmine) (×1300).

12. Cells of plants grown in culture from which gametes were obtained.

13. Plants grown in culture from which zoospores were obtained.

were maintained in wide-mouth pyrex dishes, approximately 8 cm deep, covered with a petri dish and containing 300 ml of culture solution. The culture solution comprised natural sea water, which was filtered and autoclaved, to which were added 50 ml of sterilized nutrient medium (0.1 g $NaNO_3$ and 0.02 g Na_2HPO_4 to 50 ml sterilized sea water) and 5 ml of iron citrate solution (1.0 g iron citrate to 100 ml sterilized sea water) for each 1000 ml of sea water. The culture solution was renewed every 2 weeks.

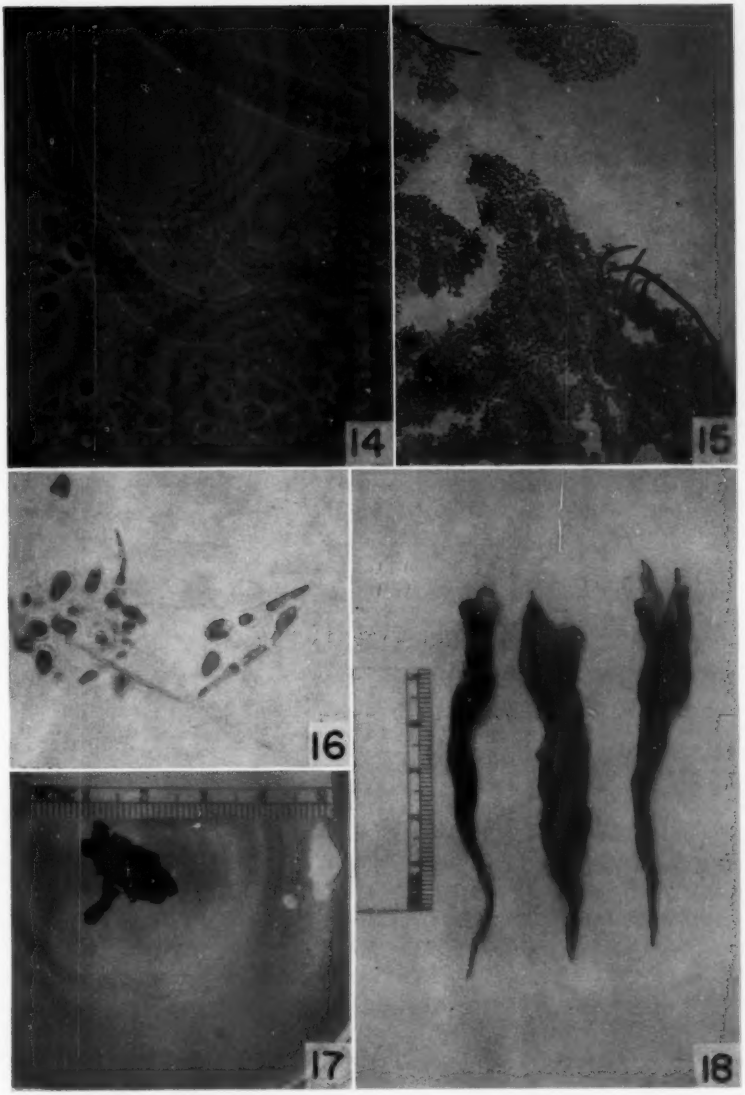
The Collinsiella-Stage in Culture

The protoplasts in the fertile surface cells, which measure 12–13.5 μ wide and 19–20 μ long at this time, were found in various stages of division (Figs. 4 and 5). The daughter protoplasts number either 8 or 16 to the cell (Fig. 2). At maturity each of the fertile cells liberates 8 or 16 biflagellate, somewhat pear-shaped motile cells (Fig. 3) through a pore measuring 4.5–5.8 μ in diameter. The motile cells are 3.5–4.8 μ in diameter, spherical in polar view, and 7.8–9.5 μ long (not including the flagella). The flagella are 14–15 μ long, equal in length, and attached at the anterior colorless end of the cell. There is a single laminate chromatophore with a single pyrenoid at the posterior end, and an eyespot is present. Early stages in the fusion of biflagellate cells were not observed and no consistent differences in size of these cells was detected. However, these aspects need to be studied further in order to determine whether these biflagellate cells behave as normal gametes, and if so whether there is isogamy or anisogamy. These points are under further study, but it is assumed the biflagellate cells are gametes, since quadriflagellate cells (planozygotes) and non-motile zygotes with two eyespots were observed in the cultures. Some evidence of a parthenogenetic development of gametes was observed.

Within a few days young germlings were abundant in the cultures. Many of them attached to the surface of the "parent" thallus but others attached to the sides and bottom of the culture vessel. The development of these germlings (Fig. 16) was studied and was found to follow that of a typical *Enteromorpha* plant (18) (Figs. 14 and 15).

The Enteromorpha-Stage in Culture

The germinating zygote at first produces a uniseriate filament (Figs. 29–34, 40–42) which soon develops rhizoidal extensions from a few of the basal cells. Frequently an extensive prostrate disc-like aggregation of filaments is produced, superficially rather similar to that described for *Blidingia* (3, 19, 30, 34) and *Monostroma* (2). However, in no instance did the later stages of development follow the pattern of development that occurs in *Blidingia*, where an "upheaval" of cells in the prostrate basal branched system occurs giving rise to an erect hollow or tubular thallus. In all instances the erect, unbranched, tubular thallus is initiated as a uniseriate filament (Fig. 16); subsequently the primary cells of the uniseriate filament undergo longitudinal divisions (Figs. 14, 43, and 44); and finally a separation in the cells occurs resulting in a hollow center (Fig. 46). However, several of these erect thalli



FIGS. 14–16. The *Enteromorpha*-stage grown in culture from the *Collinsiella*-stage; several erect juvenile *Enteromorpha* plants are shown amongst surface cells of the *Collinsiella*-stage (Fig. 14 ×200; Fig. 15 ×75; Fig. 16 ×800).
FIG. 17. *Collinsiella cava* from Izu (Shimoda), Japan.
FIG. 18. *Enteromorpha intestinalis* from a field collection on March 19, 1960.

customarily arise from the basal mass of rhizoidal cells that soon forms and these continue to arise, thus eventually resulting in a tuft of erect tubular, unbranched thalli of various sizes (Fig. 12). The mature erect thallus is solid for a short distance near the basal attachment region, where it is made up of a complex mass of intertwined rhizoidal cells embedded in a thick, mucilaginous membrane. Gradually, a short distance above this region, the membrane separates in the center to produce a hollow tube (Fig. 46). Thus, first (near the base), it appears to have several layers of cells, but distally it becomes monostromatic (Fig. 45) with the single layer of cells slightly nearer to the surface of the mucilaginous matrix or "membrane" in which the cells are enclosed (Figs. 46 and 48).

In about 9 weeks small tubular thalli 3–5 mm high had developed in the cultures (Figs. 10 and 13); in 3 months they reached a length of about 15 mm; and in 4 months they were about 20 mm long. None of these erect tubular thalli produced in culture has ever given rise to lateral branches. Occasionally the thallus becomes broadened distally (Fig. 13) and splits open at the apex. Sometimes it splits longitudinally, somewhat like a *Monostroma* (34), but this is not customary. In culture the plants are somewhat yellowish-green in color. The largest plant obtained from zygotes in culture reached a maximum length of 20 cm in 4 months; it attained a diameter of 6 mm at the apex and tapered gradually to a narrow basal attachment region. Older plants are frequently somewhat crisped and contorted in the distal portion and usually become filled with air bubbles in the swollen apex. The mature plant in culture has cells measuring 10–15 μ in diameter (Figs. 8, 9, 11, 47), somewhat angular in surface view, with no apparent regular arrangement. The "membrane" is 20–30 μ thick in cross section and the cells are centrally located or slightly closer to the outside of the "membrane" (Fig. 48). In cross section the cells are 10–15 μ in diameter and 18–20 μ high. The vegetative cells, which are uninucleate (Fig. 11), have a single laminate chromatophore with usually only one large pyrenoid (Figs. 8 and 9). Numerous starch grains usually obscure the chromatophore in the older cells.

After 2 to 3 months these tubular thalli became fertile in the distal portion. The protoplasts in cells in this region of the thallus were found in various stages of division, with 8 or 16 daughter protoplasts at maturity (Figs. 35–37). These are eventually liberated as quadriflagellate cells (or in some instances function as "aplanospores" (Fig. 39) which germinated *in situ*). These motile cells were 4.5–5 μ in diameter and 9.5–10 μ long with a laminate chromatophore, a single pyrenoid near the posterior end of the cell (Fig. 38) and a conspicuous eyespot. The flagella are equal in length, 14–15 μ long, and attached at the anterior colorless end of the cell. These flagellated cells soon settled down, germinated, and underwent the same developmental sequence recorded for the afore-mentioned parent *Enteromorpha* thallus. This sequence has occurred in three successive generations, each producing quadriflagellate zoospores. For this reason these spores are believed to be mitospores. These are comparable to the quadriflagellate zoospores that Bliding (1, 6) found were produced in culture in *E. linza*, *E. compressa*, and *E. intestinalis*, which

he refers to as "neutral spores"; that Yamada and Kanda (34) found in *Monostroma zostericola*; and that Suneson (29) reports for *M. grevillei*. Bliding (1) obtained three successive generations from such quadriflagellate zoospores in *E. linza* and four in *E. procera*. As yet no stage comparable to the *Collinsiella*-stage has been obtained in culture directly from germinating zoospores or zygotes. However, biflagellate gametes (Figs. 23 and 24) have been obtained in culture occasionally from a tubular *Enteromorpha* thallus (Fig. 12). It has not been determined whether some of these gametophytes may have arisen from parthenogenetic gametes, as has been shown to occur in *E. compressa* by Ramanathan (23). However, it would appear that most of the gametophytes obtained in culture came from germinating quadriflagellate zoospores. Parthenogenesis has been noted by several authors in different species of *Enteromorpha* as well as in other members of the Ulvaceae (1, 21), although neither Kylin (18, 20) nor Bliding (6) have observed this phenomenon in *E. intestinalis* in their studies of this species in Europe.

The same sequence of events as described above has been obtained from four different collections taken in the past 3 years and there seems little doubt that the *Collinsiella*-stage is a stage in the life history of a species of *Enteromorpha*. If the *Collinsiella*-stage is a normal sexual stage, which seems doubtful, it may not occur with regularity in the life history in an alternation of generations because of the asexual cycle indicated. However, the culture conditions may have been unsatisfactory at times for the development of the sexual cycle (or meiosis). But since normal tubular *Enteromorpha* plants (Fig. 12) producing biflagellate gametes have occasionally been obtained in the cultures, it seems unlikely that the *Collinsiella*-stage is the normal sexual phase.

In the present generally unsatisfactory taxonomic status of the extremely plastic genus *Enteromorpha* the species to which this entity is referred may be in some question. This is especially so since these observations have been made on plants growing chiefly under laboratory conditions. However, on the basis of data available, and using the morphological and anatomical criteria generally applied in the species of this genus, it would appear that the plant under consideration is *E. intestinalis* (L.) Link or a form of this species. More investigation, particularly of the kind pursued in this genus and related genera by Hartmann (15), Bliding (2, 3, 4, 5, 6, 7, and 8), Kylin (18), Moewus (21), and Suneson (29) will have to be undertaken, in order to clarify the taxonomy as well as to determine what manner of sexual

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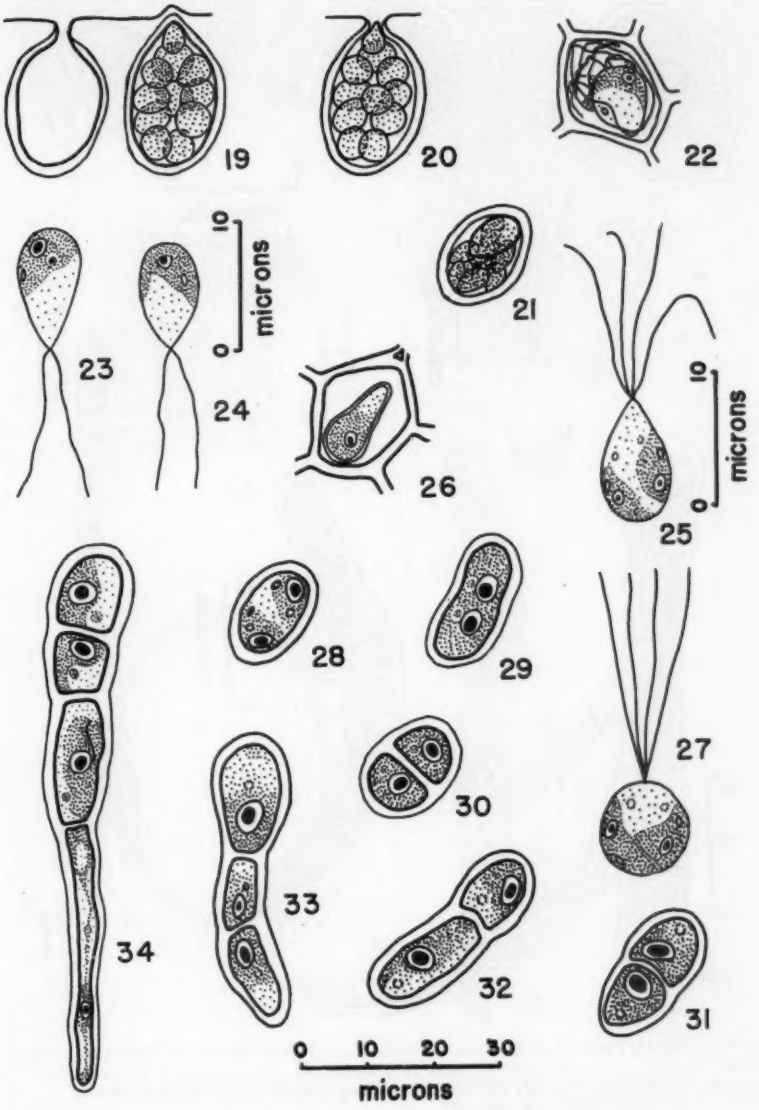
FIGS. 19–31. Gamete production and zygote germination in *Enteromorpha*.
19. Empty and mature gametangia.
20. Gametangium just beginning to liberate gametes.
21 and 22. Gametangium containing gametes of various sizes.
23 and 24. Free-swimming biflagellate gametes showing laminate chromatophore, single pyrenoid, and eyespot.
25 and 27. Planozygotes.
26. Gamete germinating *in situ* in gametangium.
28. Zygote.
29–34. Stages in germination of zygote.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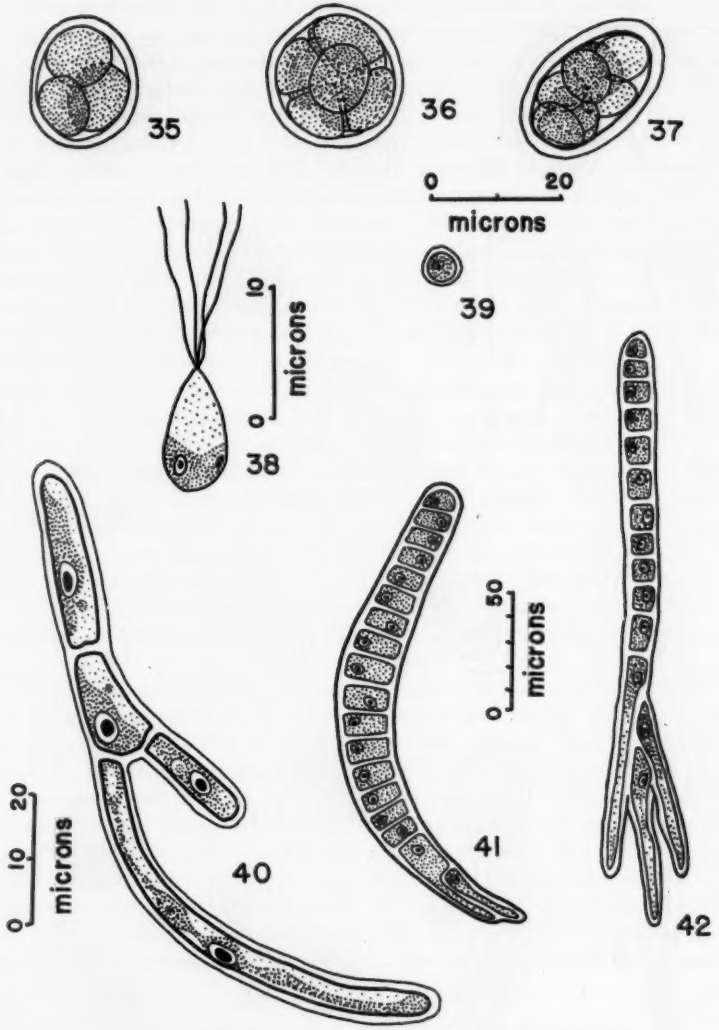
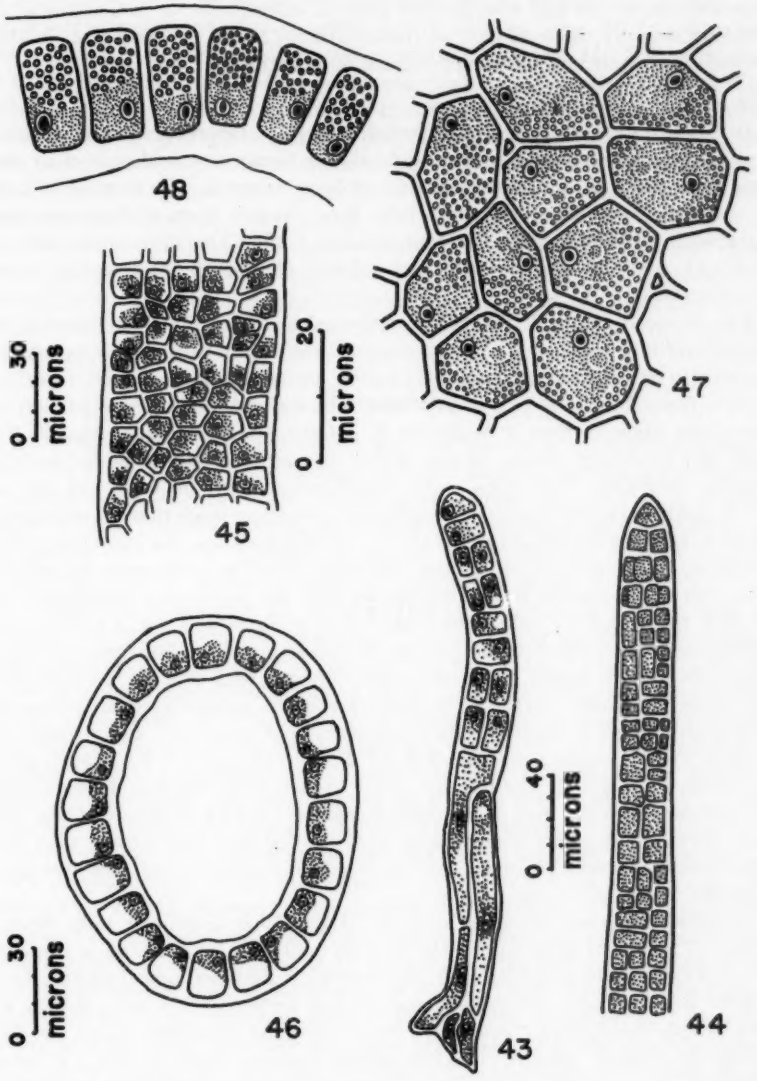


FIGS. 35–42. Zoospore production and early stages in development of the *Enteromorpha*-stage.

35–37. Zoosporangia showing various numbers of immature zoospores.
38. Quadriflagellate zoospore.
39. Aplanospore.
40. Early germling showing rhizoids forming.
41–42. Uniseriate filamentous germlings with rhizoidal base.



FIGS. 43–48. Later stages in development of *Enteromorpha* plant.
43–44. Later stages in development of germlings showing longitudinal divisions in some cells.
45. Surface view of a portion of young plant.
46. Cross section of young plant in hollow portion of thallus.
47. Surface view in mature portion of plant.
48. Cross sectional view in a mature portion of plant (outer surface at top).

reproduction occurs and whether the plant is homothallic or heterothallic. A cytological study, such as that of Ramanathan (23) in *E. compressa*, is also needed to substantiate the morphological observations.

The Enteromorpha Plant in Nature

In order to support the laboratory studies and to interpret field observations, a study of *Enteromorpha* species and related forms was undertaken in the area from which the *Collinsiella*-stage had been collected. The most abundant of these species (and at the same tidal level) were a form of *Enteromorpha intestinalis* (L.) Link and *Blidingia minima* Kylin. The very small cells of *Blidingia* and its development in culture eliminated this genus from consideration.

Fertile specimens of *E. intestinalis* collected in August 1959 produced only quadriflagellate zoospores. The specimens (Fig. 18) were up to 9 cm high, distally up to 1.5 cm broad, distinctly hollow and somewhat flattened, tapering rapidly toward the base to a narrow tube. The rather broader distal portion of this plant suggests that it might be *E. intestinalis* f. *clavata* J. Agardh. In such an extremely plastic genus which grows successfully under widely varying ecological conditions, such a difference could be expected and it seems unlikely that this form, as described, is anything more than an ecological variant. In cell size, however, and in other characteristics, the plants are very similar to those obtained in culture. The cells, 10–15 μ in diameter in surface view, are not in regular rows. In cross section, the thallus or "membrane" is 25–32 μ thick and the cells, which are 10–15 μ broad and 10–18 μ high, are slightly nearer to the outside than to the inner surface of the "membrane". The cells have a single laminate chromatophore with one or occasionally two large pyrenoids. Fertile cells produce 8 or 16 quadriflagellate zoospores each with a single laminate chromatophore, a single pyrenoid, and a posterior eyespot. The zoospores measured 4.8–7.2 μ broad and 7.2–9.6 μ long (not including the flagella). The flagella, which are equal in length and inserted at the colorless anterior end of the cell, measured 14–15 μ in length. Only the early germling stages of these plants were studied and they agreed with the observations already reported for the plants obtained in cultures initiated from the *Collinsiella*-stage.

A collection from the same area in March 1960 contained only sexual plants. The plants were up to 10 cm high and distally attained a breadth of 1–1.5 cm, tapering rapidly from the somewhat flattened apical portion to a narrow tubular base. The cells in surface and sectional view were similar in size, shape, arrangement, and cell content to those described above for the plants obtained in August. The biflagellate gametes (Figs. 23 and 24) were similar in size and shape to those described from the *Enteromorpha* plants obtained in cultures initiated from the *Collinsiella*-stage. The gametes contain a single laminate chromatophore with a single large pyrenoid at the posterior end of the cell and each has a single eyespot (Figs. 23 and 24). Early stages in the fusion of gametes has not been observed and it has not been determined whether true anisogamy, as has been reported by Kylin (18) and Bliding (1)

in *E. intestinalis*, or genotypic sex determination, as reported by Bliding (1), occurs in this material. The gametes vary in number (Figs. 19–22), size, and shape, even from the same gametangium (Figs. 21 and 22). They measure 3–6 μ in diameter and 4–10 μ in length (not including the flagella). The flagella again are equal in length, 14–15 μ long, and inserted at the anterior colorless end. Planozygotes (Figs. 25 and 27) and non-motile zygotes (Fig. 28) containing two eyespots were abundant and there was some evidence of parthenogenetic development of gametes in the cultures. Some of the gametes may even germinate *in situ* (Fig. 26).

Comparative studies of both of these are being continued in culture. However, on the basis of the morphological and anatomical characteristics and except for the slightly larger size of the plants growing in the field, these seem to be the same entity as those obtained in cultures initiated from the *Collinsiella*-stage.

Discussion

After this study had been under way for almost a year, a preliminary paper by Chihara (9) appeared dealing with the Japanese species of *Collinsiella*. From correspondence with Mr. Chihara it is understood that a more detailed paper on the Japanese species is in press.* My findings with regard to *C. tuberculata* in North America agree in some respects with Chihara's observations on the Japanese plant, but disagree in others.

Chihara (9) found that all three species (*C. tuberculata*, *C. cava*, and *C. japonica*) in Japan reproduced sexually by biflagellate gametes. He noticed slight differences in the sizes of gametes and his evidence suggested that the Japanese species are heterothallic. In all instances following fusion of gametes, which he observed, he obtained a thick-walled resting zygote similar to that described by Kunieda (17) and Yamada and Saito (35) for certain species of *Monostroma*. In *C. japonica* and *C. cava*, after several months' rest and considerable increase in size, the zygotes germinated and gave rise to quadriflagellate "zoospores". Although Chihara (9) did not observe actual liberation from the germinating zygote in the case of *C. tuberculata*, he did find comparable developmental stages subsequently in his cultures of this species. Because of the slightly different morphological characteristics of the gametophyte thallus of *C. cava* and *C. japonica* (especially the former), as well as the lack of a resting zygote in my material of *C. tuberculata* from San Juan Island, I am inclined to believe that *C. tuberculata* of North America may not be closely related to these two Japanese species. Furthermore, although *C. tuberculata* from Japan and from North America seem indistinguishable on a morphological basis, perhaps they, too, are distinct entities. The specimens of *C. tuberculata* I have examined from Japan came from Izu (Shimoda), Japan, and were

*Chihara, M. 1959. Studies on the life-history of the green algae in the warmer seas around Japan (8). On the life-history of *Collinsiella cava* (Yendo) Printz. *To be published in* J. Japan. Botany, **34** (7).

Chihara, M. 1960. *Collinsiella* in Japan, with special reference to the life-history. *To be published in* Sci. Repts. Tokyo Kyôiku Daigaku, B, **9** (140).

collected on March 10, 1955; the specimens of *C. japonica* came from Shima-Goza, Japan, and were collected on February 22, 1953.

Because of the morphological similarity of the motile stages of all of these entities they probably all belong to the Ulvaceae and in some instances at least may be stages in the life history of *Enteromorpha*; but they might also be stages in the life history of other closely related species or genera. Chihara (9) has already drawn attention to the similarity between the resting zygote of *Monostroma* and stages he obtained in his cultures of *Collinsiella*. In referring to *C. cava* (Fig. 17), which is the largest of the three species in Japan, even Yendo (37) said it appeared superficially like a young plant of *Ulva*. From a study of the material available to me, which comprised a collection made at Izu (Shimoda), Japan, on March 13, 1956, and a herbarium specimen collected by Yendo in April, 1925, at Misaki, Japan, *C. cava* (Fig. 17), in size as well as in the shape and arrangement of its cells, is the most distinctive of the Japanese species. It would seem that the conclusions to be drawn from these observations would further support the need for recognizing a single family, the Ulvaceae, to include not only *Ulva*, *Enteromorpha*, and related genera, but also *Monostroma* and *Blidingia*. Furthermore, it would seem desirable that the concept of the characteristic type of life history in this group should be modified to accept a wider variety of types in which there may be an alternation of isomorphic generations (as in some species of *Enteromorpha* and *Ulva*), or there may be an alternation of heteromorphic generations (as in some species of *Monostroma*, and in other species of *Enteromorpha* if the *Collinsiella*-stages prove to be normal gametophytes), or as in the case of other species of *Monostroma*, an alga in this family may be haplobiontic. In connection with the possible alternation of heteromorphic generations it is noteworthy that, although Yamada and Kanda (34) initially reported a life history in *Monostroma zostericola* Tilden which lacked a sexual stage and produced only quadriflagellate zoospores from the typical *Monostroma* thallus, in a recent study Yamada and Tatewaki (36) found a sexual stage in this species which occurs in the form of a small prostrate disc. The similarity of the discoid sexual thallus reported in *Monostroma zostericola* (36) to the early stages in development of the species of *Collinsiella* described by Chihara (9) is quite striking. It would be helpful to have further clarification of the subsequent development of the discoid thallus. The fact that within the one genus apparently several different types of life history are possible would further support the opinion that the Ulvaceae should be retained as a family in the order Ulotrichales rather than be placed in a distinct order, the Ulvales, as has been done by some authors.

Another possibility, which seems quite likely in the North American entity, is that the *Collinsiella*-stage may be more intimately related to (or a part of) a gametophytic generation having different morphological features at some other period in the life history. In the field my initial observations led me to think that the green layer was nothing more than the sterile basal portion of old plants of *Enteromorpha*. It now would appear that the *Collinsiella*-stage is nothing more than the last remnants of a typical gametophyte plant of

Enteromorpha intestinalis, which initially, in its erect but evanescent portion, had the typical morphology of a tubular thallus. Finally, as it matured and became fertile, eventually right to and including the very base, all that would be left of the thallus is the globose base—the *Collinsiella*-stage. The morphological detail of the two (the basal system of a mature *Enteromorpha* plant and the *Collinsiella*-stage), particularly as to their rhizoidal characteristics, are also strongly suggestive of this possibility. The fact that my material from San Juan Island came from boulders on an exposed beach, where adjacent gravel could have scoured off the erect portions of the thallus, also supports this hypothesis. It is noteworthy that the type material came from a similarly exposed region on Whidbey Island on a beach that has much the same characteristics, a mixture of boulders and gravel. Smith (28) also notes that *Collinsiella* in California grows on rocks scoured by sand. The fact that in culture there was often an intimate relationship (Figs. 14 and 15) of erect tubular thalli (which could have resulted from germination of zygotes on the surface or could have resulted from a vegetative proliferation of the gametophyte or could have resulted from parthenogenetic development *in situ* of gametes) with the *Collinsiella*-stage, would further support this possibility. The fact that the *Collinsiella*-stage occurs in nature at a time when the typical tubular sexual thallus of *Enteromorpha* is not usually found also supports this argument. However, the fact that none of the plants in culture initially produced biflagellate cells, but only quadriflagellate cells, does not support this argument. These possibilities, however, are not mutually exclusive, and in fact all might occur. The herbarium material from Waldron Island had similar young *Enteromorpha* juveniles on the surface, although these could have been contaminants. A further argument for proliferation from the base is the method by which new uniseriate filaments arise from the basal amorphous mass of a typical *Enteromorpha* thallus and gives rise to new erect tubular thalli. Bliding (1) has noted the same situation in several species of *Enteromorpha* where he reports vegetative reproduction by sprouting individuals from the basal "Haftscheibe" and Ramanathan (23) has observed the same type of proliferation in *E. compressa* var. *lingulata*. Kornmann (16) indicates a similar origin from a basal disc in *Percursaria percursa*.

The morphological and anatomical similarity of the basal rhizoidal mass of cells or "Haftscheibe" of *Enteromorpha* to the attachment region of a mature plant of *Monostroma* (33, Pl. 2, Figs. 8 *a*, *c*) and to some extent of *Ulva* (14, 35) is equally striking. For this reason, as well as the morphological similarity of the *Collinsiella*-stage and the gametes and zoospores, it would be of value to look for other possible relationships that *C. cava*, *C. japonica*, and the Japanese species referred to as *C. tuberculata* might have to species of *Monostroma*, *Enteromorpha*, and related genera known to occur in Japan. Perhaps, for example, one or all of the Japanese species of *Collinsiella* might prove to be stages in the development of an *Enteromorpha* or a *Monostroma* gametophyte or the basal portion of one of them. *C. japonica* (Fig. 17) is very similar in its vegetative characteristics to *C. tuberculata*.

Acknowledgments

I should like to acknowledge the help given by the University of California, Berkeley, in permitting me to study herbarium specimens; by Professor Yukio Yamada, Hokkaido University, Japan, who provided me with a fragment of Yendo's herbarium material of *Collinsiella cava*; and by Mr. Mitsuo Chihara, Tokyo University of Education, Japan, who sent me preserved material of *C. tuberculata*, *C. japonica*, and *C. cava* from Japan.

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STUDIES ON FUSARIUM WILT OF BANANAS

VI. VARIABILITY AND THE CULTIVAR CONCEPT IN FUSARIUM OXYSPORUM f. CUBENSE[1]

B. H. WAITE AND R. H. STOVER

Abstract

The range in cultural variability of *Fusarium oxysporum* f. *cubense* on potato dextrose agar was studied. Several hundred isolates obtained from diseased varieties of *Musa* were categorized into four morphological groupings or cultivars. These were termed 'Sporodochial', 'Ropy', 'Cottony Alba', and 'Slimy Pionnotal'. The morphological cultivar 'Sporodochial' was further divided physiologically into 'Odoratum' and 'Inodoratum' cultivars. Clones producing sporodochia were the parental forms from which all other cultural types could arise by mutation. Mutants did not revert to their parental types. A system is proposed for cataloguing clones of plant pathogenic *Fusarium* species. Based on morphological characters, the catalog is intended to serve as a collection of data which provides also for the categorizing of cultivars.

Introduction

During the early 1920's extensive investigations on the taxonomy and distribution of fusaria were conducted by Reinking and Wollenweber in Central America (5, 6, 23). Although the banana wilt organism (*Fusarium oxysporum* f. *cubense* E. F. S. Snyd. & Hans.) was the principal fungus under study, isolations from soil and plant material resulted in a collection of numerous other fusaria. A conference, attended by Wollenweber, Sherbakoff, Reinking, Johann, and Bailey, was held at the University of Wisconsin to discuss, identify, and compare cultures of the tropical fusaria as well as those from the United States and Europe. Descriptions of spore forms and types, sclerotia, sporodochia, and pionnotes were published. Color of conidia, aerial mycelia, and stroma were considered in culture identification as well as odors and mycelial characters (22). The morphological characters which had earlier been defined by Wollenweber (21) have continued to be of importance in any new consideration of *Fusarium* taxonomy and nomenclature. Wollenweber and Reinking established a taxonomic system in which differences in spore size, sclerotia formation, chlamydospore formation, color, and odor were used as characters to define about 65 species and 55 varieties (24). Snyder and Hansen (9, 10, 11), noting basic similarities among many of these species, later simplified the taxonomic system and reduced the number of *Fusarium* species to nine. No varieties were recognized. The authors nevertheless recognized the capacity of individual cultures originating from a single spore to exhibit variability while at the same time retaining characters of the species.

The influence of cultural conditions upon the morphological characters used in the identification of fusaria has long been recognized. As stated by

[1]Manuscript received May 10, 1960.
Contribution from Plant Pathology Department, Vining C. Dunlap Laboratories, Tropical Research Department, Tela Railroad Co., La Lima, Honduras (subsidiary of United Fruit Company).

Snyder and Hansen (8) "Conditions of culture, which are reflected in important degrees of growth obtained, include the purity of culture, kind of medium, method of transfer, frequency of transfer, past cultural history of the fungus, age of culture, amount of light in the environment, temperature, and variability of the fungus."

The foregoing investigations all dealt with problems in *Fusarium* taxonomy, i.e., the definition of species differentiated on the basis of cultural morphology. The term *forma specialis* was applied at a subspecies level to those species biologically or pathologically distinct, a concept independent of morphology.

All aspects of variation and speciation in *Fusarium* were reviewed by Snyder and Hansen in 1954 (13). In this publication they also make first mention of the concept of "horticultural varieties", a term devised to meet the problem in handling distinctive clones of *Fusarium* species. The term "horticultural variety" has been widely accepted in the nomenclature of higher plants where individuals of a single species may have certain distinctive features which set them apart from other individuals of the species. The analogous situation in fungi prompted the horticultural, or, preferably, "cultivar" concept for *Fusarium*. For example, six cultivars were proposed for non-pathogenic types of *F. roseum*, using species names formerly retained in the Wollenweber and Reinking system of classification. In the same manner, a cultivar was recognized for *F. oxysporum* and also for *F. solani*. Two cultivars were proposed for *F. episphaeria* and a single cultivar suggested for *F. moniliforme*. All cultivars proposed in this publication were based on cultural characters (14).

The present paper describes the behavior of *Fusarium oxysporum* f. *cubense* in culture and relates this to present concepts in *Fusarium* variability and nomenclature.

Methods

The terms isolate, clone, and race, as they apply to the fungus, were defined previously (16). However, since the present study is a further elaboration of the cultivar concept, the term cultivar is redefined in greater detail. In addition, other terms used in this paper are defined:

Cultivar:	"The term cultivar (variety) denotes an assemblage of cultivated individuals which are distinguished by any characters (morphological, physiological, cytological, chemical, or others) significant for the purposes of agriculture, forestry, or horticulture, and which, when reproduced (sexually or asexually), retain their distinguishing features" (3).
Sporodochium:	A mass of branched conidiophores bearing macroconidia tightly placed together upon a stroma or mass of hyphae (1).
Pionnotes:	A slimy or gelatin-like layer of conidia (1). Conidiophores producing macroconidia are simple rather than highly branched as in sporodochia.
Mutation:	Any spontaneous genotypic change in a homokaryon culture derived from a single spore which results in heritable morphological or physiological differences from the parent culture.

To determine the range in cultural variability of *F. oxysporum* f. *cubense*, several hundred isolates were obtained from 20 banana-producing countries. The fungus was recovered from diseased rhizome or pseudostem tissue of

PLATE I

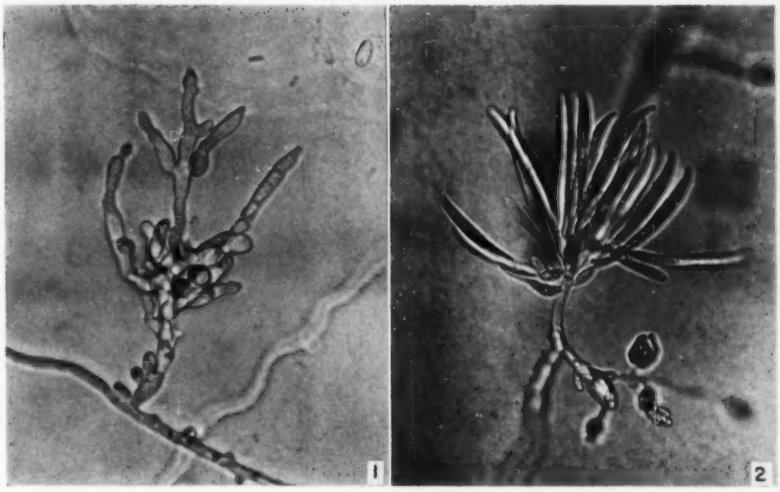

FIG. 1. An early stage in the development of the sporodochium showing a highly branched conidiophore with macroconidia removed.

FIG. 2. A fully formed young sporodochium with macroconidia attached. Note the false heads of microconidia produced beneath.

different edible varieties of *Musa*, using the method described by Stover and Waite (15) or by placing bits of dried diseased plant tissue on natural media following the method of Hansen and Snyder (12). In some cases agar cultures were obtained from foreign sources. Following initial isolation, cultures of single spore origin were maintained on test tube slants of potato dextrose agar, and in sterile soil (4). All subsequent transfers for cultural studies were of single spores.

Potato dextrose agar (PDA) was prepared using the following method: 20 g of agar was washed and filtered. Two hundred and fifty grams of potatoes were washed and thinly sliced but not peeled. The potatoes were steeped in a small amount of water at about 60° C for 1 hour, then filtered through four layers of cheesecloth. The agar and potato solutions were mixed and 20 g of dextrose was added. The volume of mixture was brought up to 1 liter with distilled water. The pH was adjusted to 6.2 with NaOH. Thirty milliliters of melted PDA was used per test tube (200×25 mm), and autoclaved for 15 minutes at 15 lb pressure before the tubes were slanted.

Cultures were maintained at fluctuating room temperature (20–27° C) in strong daylight but not in sunlight. If artificial light was used, 175–200 foot-candle intensity from fluorescent lamps about 12 hours per day was found satisfactory for development of colony morphology. It required approximately 4 to 6 weeks for all the cultural characteristics to be fully expressed on PDA slants. Certain clones were also grown on steamed rice for 2 weeks in 125-ml flasks for the determination of odors.

Selected clones were tested for pathogenicity in banana plants grown under field conditions and in large tanks. The seedling pathogenicity test described by Stover (17) was also used.

Results

Under the conditions described above, individual clones of *F. oxysporum* f. *cubense* races 1 and 2 (18) developed different cultural appearances and developed at varying rates. Nearly all clones isolated from naturally diseased edible varieties of *Musa* developed a thallus of abundant, floccose, fine aerial mycelia and eventually produced sporodochia within 2–4 weeks following transfer to PDA (Fig. 3). A few produced sporodochia only after the agar slant began to dry and shrink. Clones producing sporodochia exhibited considerable range in the amount produced and their appearance. Sporodochia produced masses of macroconidia that appeared as wet, yellow or flesh-colored beads on the colony surface (Fig. 4), or the sporodochia and macroconidia remained embedded in mycelia. Occasionally large patches of wet spore masses were evident when sporodochia were abundant. Macroconidia were also produced on simple conidiophores in those cultures with sporodochia. Sclerotia were characteristically present.

In addition to the predominant sporodochia-forming cultures, a few isolates were obtained from diseased tissue which never produced sporodochia. Macroconidia were produced in slimy pionnotes or sparsely in abundant aerial

mycelia. Similar non-sporodochial types were obtained by culturing from mature colonies of the sporodochia-forming cultures. Thus non-sporodochia-forming cultures arose by mutation from those forming sporodochia. These non-sporodochial cultures were of three types.

The first and most common non-sporodochial cultures to appear as a result of mutation were characterized by abundant aerial mycelia, generally of a ropy or coarse nature when contrasted with the finer, floccose mycelia of the parent culture (Fig. 3). They were often highly pigmented purple or bright pink. Macroconidia were produced on simple conidiophores. To some degree these macroconidia were in pionnotes partly embedded in the substrate. Sclerotia were absent in cultures with coarse, ropy mycelia.

A second type of non-sporodochial culture occasionally was derived by mutation from cultures producing sporodochia and from naturally diseased banana tissue. These mutants developed an abundance of fine white or pale pink aerial mycelium and were designated the 'Cottony Alba' type (Fig. 5). Macroconidia were few and were produced on simple conidiophores. Sclerotia were never observed. These fine white mycelial cultures were relatively stable and only rarely mutated to the coarse, ropy mycelial cultures described above.

The third group of non-sporodochial cultures, derived as a result of mutation, produced scant or no aerial mycelia (Fig. 3). Macroconidia were produced in slimy pionnotal masses. These appressed cultures were often highly pigmented. Sclerotia were lacking. Slimy pionnotal cultures did not mutate to the other cultural types described.

In all isolates studied, the clones producing sporodochia were observed to be the parental forms from which other cultural types could arise by mutation. This mutation process was unidirectional. Clones in the ropy group almost always originated from the sporodochial group. Most ropy clones readily mutated to the slimy pionnotal clones. The white or pale pink mycelial clones ('Cottony Alba') arose as mutants from the sporodochial group. They were stable and only rarely mutated to the ropy group. The rate of mutation from one cultural group to another depended on the mutation potential of the individual clone as well as cultural conditions, particularly the age of the culture. Some clones mutated rapidly while others remained comparatively stable.

The sporodochial group of cultures was the predominant morphological form in nature; it was possible to divide this group further on the basis of physiology into 'Odoratum' and 'Inodoratum' cultivars (16). The former

FIG. 3. An example of the variability pattern of *F. oxysporum* f. *cubense* isolated from naturally infected banana tissue. Left to right: a sporodochial clone; a sporodochial clone showing patch mutations; a ropy mycelial clone; a ropy mycelial clone which has mutated towards the slimy pionnotal type; a slimy pionnotal type.

FIG. 4. A clone of *F. oxysporum* f. *cubense* producing bead-like erumpent masses of macroconidia from sporodochia on the colony surface.

FIG. 5. Four clones of the 'Cottony Alba' type culture showing an abundance of fine white aerial mycelia.

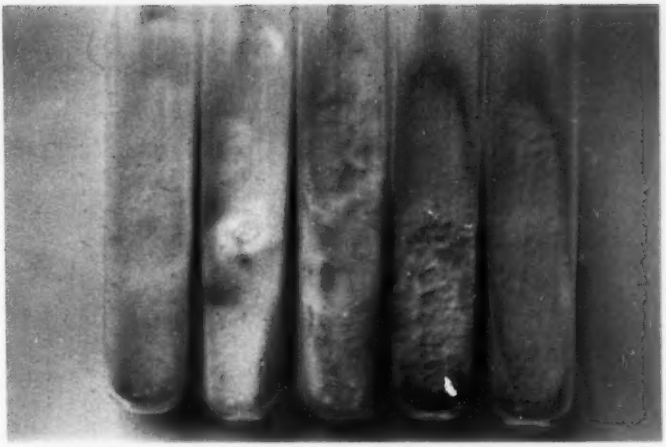

FIGURE 3.

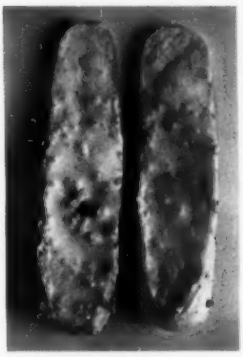

FIGURE 4.

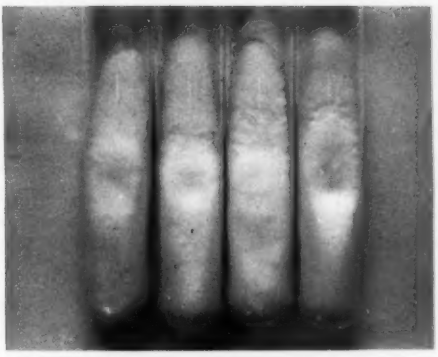

FIGURE 5.

TABLE I

Distinguishing characteristics of the 'Odoratum' and 'Inodoratum' clones within the 'Sporodochial' cultivar of *F. oxysporum* f. *cubense*

Substrate	'Odoratum'	'Inodoratum'
Potato dextrose agar slants at 2–4 weeks	Colony color varies from gray to pale purple with zonation and pigment in substrate (dark clones) to a light colored, faint pink to cream colony with weak or no zonation or pigment in substrate (light clones). Slight to pronounced sharp odor from about 10 to 20 days. Sporodochia embedded (dark clones) to erumpent (light clones)	White, coarse-appearing, dense mycelium; varying shades of purple in some clones; varying amounts of purple pigment produced in substrate; no zonation. Usually no odor and never sharp. Sporodochia embedded and erumpent
Steamed rice at 2 weeks	Strong and sharp odor; white to pale pink mycelium; surface of mycelial mat bumpy; pigment production pale pink to purple and relatively poor	Mild "fruity" odor or no odor; white to rosy-pink mycelium; strong pink to deep purple pigment production in most clones

were distinguished mainly by the production of a strong sharp odor on rice (Table I). This particular odor was not found among clones of the 'Inodoratum' cultivar, some of which produced a mild "fruity" odor differing from that of 'Odoratum' clones. The strong sharp odor produced on rice by 'Odoratum' clones was never detected from non-sporodochial cultures. Within each group, there was a considerable range in colony characteristics such as color and zonation of the thallus, pigment production in the medium, and sporodochia production (Table I). Cultures were obtained from 'Odoratum' clones that did not produce the odor characteristic of this cultivar, whereas 'Inodoratum' clones never yielded cultures that produced the characteristic odor of the 'Odoratum' cultivar. It appears, therefore, that the 'Inodoratum' clones were derived from the 'Odoratum' by mutation.

Discussion

Cultivars of Fusarium oxysporum f. cubense

On the basis of cultural behavior all clones of *F. oxysporum* f. *cubense* may be categorized into cultural groupings or 'cultivars'. The four main morphological cultivars recognized are 'Sporodochial', 'Ropy', 'Cottony Alba', and 'Slimy pionnotal'. The distinguishing features of these cultivars are summarized in Table II. These cultivar designations convey a definite description of certain cultural and morphological aspects of the particular isolates or clones under consideration. Where the 'Sporodochial' cultivar is involved, 'Odoratum' and 'Inodoratum' were recognized as physiological cultivars within this morphological group of clones. Odors characteristic of the 'Odoratum' cultivar were not detected in any of the mutants which arose from the 'Sporodochial' cultivar. Clones undergoing transfers in culture sometimes change slightly in appearance but may not move completely out of one cultivar into another. This situation is particularly evident for those clones within the 'Sporodochial' and 'Ropy' cultivars. Occasionally mutations may

TABLE II

Distinguishing features of the four major morphological cultivars of *F. oxysporum* f. *cubense* on potato dextrose agar after 3 weeks

Character	Cultivar			
	'Sporodochial'	'Cottony Alba'	'Ropy'	'Slimy Pionnotal'
Sporodochia	Present, erumpent or submerged	Absent	Absent	Absent
Pionnotes	Present	Few present or absent	Present	Present and abundant
Aerial mycelium	Abundant and floccose	Very abundant; fine and cottony	Very abundant; coarse, ropy and often raised in tufts	Scant and appressed
Colony color	Variable from near white to pink or purple	White or pale pink	Often highly pigmented	Often highly pigmented
Sclerotia	Present in variable amounts	Absent	Absent	Absent
Mutability	Some clones stable, others unstable, mutating to ropy and slimy pionnotal	Stable	Usually highly unstable, mutating to slimy pionnotal	Stable

result in a borderline case where it is difficult to place a clone in a definite cultivar. In these cases the investigator must use more critical judgment based on experience in selecting the proper cultural group for the clone in question.

Hansford (2) and Wardlaw (19) isolated morphologically distinct types of *F. oxysporum* f. *cubense* from diseased banana tissue and described cultures derived from single spores or hyphal tips which can be grouped into one of the cultivars described in this paper. Wellman and Blaisdell (20), working with the tomato wilt *Fusarium*, observed cultural variability, and the extent and direction of mutation. Their results indicated a pattern of mutation in culture similar to that of *F. oxysporum* f. *cubense*. According to Snyder and Hansen (9), all vascular wilt fusaria are morphologically similar and it is possible to distinguish the *forma specialis* only by pathogenicity tests. It is not unlikely, therefore, that all vascular wilt fusaria may show the same mutational and cultural pattern. More recently Schneider (7) observed a similar pattern in *F. avenaceum* (Fr.) Sacc. (*F. roseum*).

Catalog of Fusarium Clones

A detailed study of the culture and pathogenicity of fusaria requires that the worker have as complete a history and description of the fungus as is possible. To facilitate recording of this information a form is proposed for cataloguing of individual *Fusarium* clones. An example, with the description of a clone of *F. oxysporum* f. *cubense*, is shown below. This catalog, although

based on studies with *F. oxysporum* f. *cubense*, is designed to record information for any plant pathogenic *Fusarium* species. Similar forms are already in use by bacteriologists. The form is divided into five sections concerned with the history of the isolate, cultural descriptions for the individual clones, physiological traits, host range and habitat, and other informative remarks. When an isolate is obtained, some preliminary information can be recorded. Further work with the culture enables the investigator to fill in more spaces in the form until eventually a complete record is obtained. Some workers may be interested only in certain characteristics such as cultural description, while others can later supply other information. In this manner the catalog eventually builds up a collection of data to complete the description for individual *Fusarium* clones. The catalog then serves as a readily available summary of information for any particular clone which might be retained for studies at some future date. It is designed broadly enough to fit any worker's needs and may be supplemented, if necessary, with more categories to describe cultivars. By completing the "Cultural Description" category the cultivar type can be determined. This enables the worker to have a single term available for describing certain characteristics of a particular clone. When the clone is studied at some future date the worker will also be able to determine any mutational changes which might have occurred in storage by comparing his most recent cultures with the original clonal description.

To establish some degree of standardization, certain cultural procedures are also recommended in the catalog form. Potato dextrose agar, prepared in the manner described above, is suggested as the medium for sporulation and other cultural features of fusaria. Steamed rice is most suitable for determining 'Odoratum' and 'Inodoratum' clones in the 'Sporodochial' cultivar of *F. oxysporum* f. *cubense*. An understanding of clonal variation and stability in culture is basic to the whole catalog concept. Obviously it is impossible to determine or control minor changes due to variations in cultural practices. To avoid the loss of clones in culture through mutations some method of storage that reduces the amount of variation is necessary. Clones may be preserved in soil (4) or by using some other method which should be indicated in the 'History of the Isolate'. Single spore transfers should be made at intervals throughout the period of storage to insure maintenance of the original clone and to determine changes as a result of mutations.

A SUGGESTED CATALOG OF *Fusarium* CLONES

File No._____

Fusarium species: *oxysporum* Classification* Si

Forma specialis *cubense* Classified by B. H. Waite

Race ? Date February 25, 1960

.....

History of Isolate

1. Worker's name B. H. Waite — Worker's Cult. No. or Designation H
2. Date isolated October 15, 1959 — Worker's classification 'Sporodochial' 'Inodoratum'
3. Origin: Country Honduras — State, county, etc. Choloma
4. Host species *Musa*—plantain — Variety 'Chato' 'Bluggoe'
5. Source: Plant part rhizome Soil _____ Water _____ Other _____
6. Cultural substrate PDA
7. Methods of storage† Sterile soil: transferred by single spores every 6 months
8. Date single spored October 20, 1959 — By whom B. H. Waite
9. Pathogenicity tested on Chato — Date December 1959
10. Distribution North Coast Honduras
11. Remarks _____

Culture Description†
(Underline or fill in)

12. Macroconidia: in sporodochia, in pionnotes, scattered in mycelium _____
    Sporodochia: abundant, moderate, few; color of conidial masses flesh
13. Aerial mycelium: cottony, ropy, appressed, abundant, sparse, even, uneven, _____
14. Color of aerial mycelium: white, very pale pink, purple, _____
15. Color of submerged mycelium: pale purple Substrate color: purple
16. Sclerotia: abundant, few, absent, large, small, _____
17. Chlamydospores: few, abundant, PDA
18. Zonation: absent, weak, strong _____
    Medium used for 12 to 18: PDA
    Age of culture: 5 weeks Describer B. H. Waite
19. Odor on rice: absent, weak, strong, sharp, mild _____
    Pigmentation on rice: strong purple
    Age of culture on rice: 2 weeks Describer R. H. Stover
20. Other features: None

Physiological Traits

Describer _____

Not studied

PATHOGENICITY AND HOST RANGE

Describer R.H. Stover

1. Host range
   Attacks certain ABB varieties of *Musa acuminata* × *Musa balbisiana* but not AAA varieties of *Musa acuminata*
2. Virulence
   Highly virulent on plantain variety 'Chato' or 'Bluggoe'

. . . . . .

REMARKS

Most 'Sporodochial' cultivars produced mutant 'Ropy' clones. 'Ropy' clones are relatively stable.

. . . . . .

*Cultivars and Symbols: 'Sporodochial' = S; 'Ropy' = M; 'Slimy Pionnotal' = P; 'Cottony Alba' = A

Clones with a strong sharp odor on rice ('Odoratum'), and without a strong sharp odor on rice ('Inodoratum') may be indicated with the letters o and i; e.g., So and Si mean a sporodochial cultivar with strong sharp odor and a sporodochial cultivar without strong sharp odor respectively. Other cultivar symbols may be devised.

†Procedures:

Media

For sporulation and other cultural features incubate in diurnal diffuse light at room temperature or at about 200 ft-candles for 12 hours per day, using PDA as prepared in this paper.

For the expression of pigments and odor, incubate on steamed rice, prepared as follows: add 7.5 g of clean dry rice and about 25 ml of distilled water to a 125-ml flask; plug with cotton and autoclave at 15 lb for 15 minutes. Incubate cultures at room temperature in diurnal diffuse light for 2 weeks.

Chlamydospores

May be determined on agar media or in water cultures. In No. 17, indicate the method used.

Method of storage

On agar slants, in soil, under oil, etc.

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